

524/518

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



11 FEB 2005



(43) International Publication Date
26 February 2004 (26.02.2004)

PCT

(10) International Publication Number
WO 2004/016276 A1

- (51) International Patent Classification⁷: **A61K 35/23**,
A61P 13/12 [GB/GB]; Intercytex Limited, Grafton Street, Manchester
M13 9XX (GB).
- (21) International Application Number:
PCT/GB2003/003575
- (22) International Filing Date: 14 August 2003 (14.08.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0218916.5 14 August 2002 (14.08.2002) GB
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- (81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,
SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: KIDNEY FORMATION

(57) Abstract: In one aspect, the invention provides a chimeric kidney located on or near a large blood vessel in the abdomen of a mammalian host, in which the chimeric kidney is capable of producing urine. In another aspect, the invention provides a method of increasing the nephron mass of a mammalian recipient comprising implanting a metanephros of an embryonic, preferably non-human, mammalian donor on or near a large blood vessel of the recipient.

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Kidney Formation

The present invention relates to chimeric kidneys and methods for increasing the nephron mass of a recipient by
5 implantation of embryonic kidney rudiments.

Kidneys are excretory organs that maintain chemical and physical constancy of blood and other body fluids by removing superfluous water and materials which are
10 biologically useless or toxic. The kidney is comprised of a number of functional units called nephrons. Each nephron has a complex structure with two main parts: the glomerulus and the renal tubule. The glomerulus comprises non-anastomosing capillaries located in the cortical substance and is
15 surrounded by the capsule of Bowman, which is part of the renal tubule. The renal tubule is located partly in the cortical substance and partly in the medullary substance and terminates into a collecting tubule which opens into a ureter. The ureter opens into the urinary bladder.

20 During embryogenesis, the rudiments of the permanent kidneys, the metanephroi, appear during the fifth week of gestation in humans, during day 12 of embryonic rat development and during day 11 of embryonic mouse
25 development. Humans develop a full complement of nephrons by approximately 35 days of gestation. However, in rodents nephrogenesis continues for the first 2 weeks following birth, when nephrons continue to develop from a nephrogenic zone located at the periphery of the kidney. Once mammalian
30 renal development is complete, no new nephrons are formed under any conditions.

Loss of kidney function resulting in end-stage renal failure is a major clinical problem with a wide variety of causes
35 including diabetes, immune mediated inflammatory disease, hypertension, urinary tract infections and genetic predisposition (polycystic kidney disease). In the UK, the

cost of renal replacement therapy (RRT) consumes 2% (£1.2 Billion) of the NHS Budget with a predicted rise to 3% within 5 years. In the US, the annual cost is estimated to be in excess of \$15 billion.

5 During 1999 in the UK, approximately 5350 people started renal replacement therapy for the first time, joining 31,147 patients already receiving treatment. Without prompt suitable treatment, death is inevitable. Currently, 52% of
10 patients receive dialysis treatment in hospital or at home using a haemodialysis machine or peritoneal dialysis, whereas 48% of patients receive a kidney transplant the majority from an unrelated donor. All dialysis methods have their specific problems and while these techniques are life
15 sustaining, patients lead a poor quality of life and are at high risk of death from cardiovascular disease. While transplantation is regarded as a superior replacement method, at present it requires a major surgical procedure and lifelong immunosuppressive therapy with significant
20 increased risk of death from serious infection and cancer.

The half-life of a kidney transplant is currently about 8 years, which means that young patients face the potential ordeal of 3 or 4 kidney transplants during their lifetime.
25 However, transplantation is greatly limited by a severe shortage of donor organs, which is unlikely to change in the foreseeable future. Currently 5500 people in the UK are on the waiting list for kidney transplantation and yet only 1500 kidneys are available annually for transplantation.
30 This organ shortage is replicated throughout the developed world. In the developing world, renal failure is often a death sentence as the current technology is unavailable or prohibitively expensive. Globally, the clinical need for the development of alternative renal replacement therapies is
35 enormous.

Use of embryonic kidneys to form chimeric kidneys is

disclosed in USP 5,976,524 (Hammerman), which is incorporated herein by reference in its entirety. Increased metanephron mass is achieved by transplantation of embryonic kidney metanephroi (E14 or E15 in rats) implanted next to the omentum or under the renal capsule.

Hammerman's approach involves transplanting embryonic day 15 (E15) rat metanephroi into the omentum of an adult host, the metanephroi are then left to develop for approximately 4 weeks before the metanephric ureter is connected to the adult ureter using an end-end anastomosis. Typically, this results in approximately 3% renal function in the rat recipients compared with normal function of two kidneys. At best, 5-7% renal function compared to normal is achieved. Using the Hammerman technique, development and vascularisation of new kidney structures is relatively slow (about 6 days) and an inflammatory response, which requires an immunosuppressive regime and/or anti-inflammatory approach, is observed in our hands even for allogeneic outbred transplants.

Current therapies for end stage renal failure such as dialysis offer around 10% renal function. Typically, clinicians put a patient on dialysis at 7% of normal renal function. It is thought that to produce an effective product, the renal function of kidneys formed from transplanted metanephroi should be improved to a level at least as effective as dialysis. For comparison, approximately 40% of normal renal function is expected from allograft kidney transplantation. There is therefore the need for a more effective chimeric kidneys and a more effective method to produce kidneys from transplanted metanephroi.

According to a first aspect of the present invention, there is provided a chimeric kidney located on or near a large blood vessel in the abdomen of a mammalian host, in which

the chimeric kidney is capable of producing urine. Alternatively, the chimeric kidney is located on or near adventitia of the large blood vessel. The chimeric kidney is preferably located in the peritoneal cavity of the host.

As described in further detail below, the chimeric kidney of the present invention has improved characteristics compared with those known in the prior art, including more efficient urine production.

The term "chimeric kidney" is used herein to describe a kidney comprising inseparable tissue from both donor and recipient origin. The kidney tissue therefore comprises cells of different genotypes. The chimeric kidney is typically bounded by a renal capsule. The chimeric kidney may contain a single interconnecting branching network of tubules forming a collecting duct system which is formed by the development and growth of a ureteric tissue of the kidney precursor (for example, a metanephros) in the host and which is preferably functionally linked to the host's urinary system via the ureter of the chimeric kidney.

By "near a large blood vessel" means preferably within 10 mm of the large blood vessel in a human or a relative similar distance in other mammalian hosts. The chimeric kidney may be located in a pouch formed in the retroperitoneal fat adjacent the large blood vessel.

A large blood vessel may be defined by cardiac output (see below).

The chimeric kidney may be in fluid communication with the host's large blood vessel via the hilum of the chimeric kidney. Advantages of contact through the hilum includes more efficient filtration.

The chimeric kidney may be in fluid communication with the host's large blood vessel via vasculature which originates from the large blood vessel and which enters the chimeric kidney within an area defined by a frustoconical shape having a cone angle of less than 90 degrees and an apex at the chimeric kidney. In comparison with prior art chimeric kidneys, the present invention allows more direct fluid communication (for example, blood supply and/or drainage) between the chimeric kidney and the host.

The chimeric kidney may have developed a ureter to facilitate externalisation of urine.

Urine produced by the chimeric kidney may be excreted via the host's ureter.

The chimeric kidney may have been formed from an embryonic metanephros, which may be implanted (i.e. transplanted) into the host (also referred to herein as "recipient"). The chimeric kidney may thus be an organ comprising cells both of donor origin derived from an implanted embryonic metanephros (which at the time of implantation is largely, for example approximately 95%, embryonic mesenchyme and ureteric bud) and of host origin including but not limited to endothelial cells. There is typically a large increase in size and development of the transplanted metanephros and development of glomeruli to form the chimeric kidney. There is also typically the development of vasculature in the chimeric kidney. Embryonic metanephros used for implantation preferably do not have glomeruli and do not contain arteries, arterioles, veins or venules at the time of transplantation into the host. However, discrete clusters of endothelial cells may be present in embryonic metanephros used for implantation.

The embryonic metanephros or embryonic metanephroi used are preferably porcine. The host is preferably human.

The large blood vessel is preferably the renal vein (see Experimental section below for advantages of this site). Alternatively, the large blood vessel may be the renal artery.

In a further aspect of the invention there is provided a chimeric kidney multiplex or chimeric kidney assembly comprising two or more chimeric kidneys as defined herein.

The chimeric kidney multiplex may comprise an interconnecting manifold linking the two or more chimeric kidneys. The interconnecting manifold may have formed after development of the two or more chimeric kidneys.

The term "chimeric kidney multiplex" describes a set of separate but proximal chimeric kidneys. The multiplex may have developed following implantation of embryonic metanephroi in close proximity to one another, for example such that ureters developed by the embryonic metanephroi are aligned with each other. Each chimeric kidney within the multiplex may have a separate blood supply tapping from the large blood vessel, for example the large blood vessel on or near which the originating metanephroi were implanted. Each chimeric kidney within the multiplex may be bound by its own renal capsule and develop its own collecting duct system and ureter. The ureters of the chimeric kidney multiplex may be joined, e.g. via anastomosis, to form an interconnecting manifold which may drain into the host urinary system.

A chimeric kidney multiplex formed from the two or more embryonic metanephroi has a greater chance of efficient functioning.

In a further aspect of the present invention, there is provided a method of increasing the nephron mass of a mammalian recipient comprising implanting a metanephros of an embryonic mammalian donor on or near a large blood vessel

of the recipient under conditions that allow the metanephros to become vascularised.

The method of the current invention allows for improved development in a number of respects. For implantation into outbred mammals, in particular, these include: (i) more glomeruli; (ii) larger, more mature glomeruli; (iii) an improved collecting duct system demonstrating superior tubulogenesis; (iv) improved vasculature; and (v) a reduced inflammatory or immune response (compared for example with Hammerman's approach). This improved development could not have been expected from the prior art as the environment of embryonic development of metanephroi more closely resembles the Hammerman transplantation site (at least for the renal capsule if not the omentum) than that of the present invention. A reduced inflammatory or immune response may also be regarded as unexpected as a large blood vessel might be expected to increase the transport of inflammatory molecules to the site of transplantation.

In a preferred aspect of the invention, the metanephroi are implanted below the diaphragm of the host, for example in the peritoneal cavity. The large blood vessel should preferably be below the diaphragm so that the thorax of the recipient is not opened during implantation of the metanephros. In this aspect of the invention, the following aortic branches are excluded as sites for metanephros transplantation: pericardial, bronchial, oesophageal, intercostal and superior phrenic arteries. The advantages in metanephric development for these sites compared with the sites of this aspect of the invention may in principal be shared, but there could be subsequent technical problems with connection of the transplanted graft ureter to the host ureter.

By "near a large blood vessel" means preferably within 10 mm of the large blood vessel in a human or a relative similar

distance in other mammalian recipients. The metanephros may be implanted in a pouch formed in the retroperitoneal fat adjacent the large blood vessel.

- 5 The vascularised metanephros may form a chimeric kidney that produces urine and develops a ureter that facilitates externalisation of the urine.

10 Our results show that metanephroi implanted at or near a large blood vessel are more efficient at urine production, for example as evidence by the presence of cysts or sacs containing urine ("urine cysts"). Urine cysts may comprise connective tissue and kidney epithelial tissue. Urine cysts may be connected directly to the recipient's ureter for
15 externalisation of urine.

The resulting chimeric kidney may be heavily vascularised and have improved development compared with implantation of metanephroi next to the omentum. In terms of gross
20 morphology, the chimeric organs produced by the present method are shown in the Experimental section below for outbred animals to resemble a small kidney. There appear to be differences also at the histological level. The chimeric organs produced by transplantation to the new site are shown
25 in the Experimental section below to have more glomeruli, part of the functional unit of filtration the nephron. Greater functionality as measured by standard techniques such as inulin clearance may thus be achieved.

30 A feature of kidney formation from a transplanted metanephros is that the metanephros is a bundle of cells which develops into a mature, functional organ. The teachings from prior art disclosures related to transplantation of cells which are already functional, for
35 example the pancreatic cells in USP5,629,194 (Dinsmore), are thus not directly transferable to development of transplanted metanephroi.

The large blood vessel may be the aorta or a branch of the aorta. Preferably, the branch may be a primary branch from the descending abdominal aorta. For example, the large blood vessel may be a renal artery, an iliac artery, a gonadal artery (spermatic in the male, or ovarian in the female) or an hepatic artery.

Alternatively, the large blood vessel may be the vena cava or a branch of the vena cava. For example, the large blood vessel may be a renal vein, an iliac vein, a gonadal vein (spermatic in the male, or ovarian in the female) or an hepatic vein. The renal vein is particularly preferred.

The large blood vessel may also be defined by percentage of total cardiac output. For example, the large blood vessel may be one via which the chimeric kidney, for example when fully developed, may derive blood proportionate to the requirements of the chimeric kidney and up to 25% of total cardiac output at rest. A kidney typically uses 25% of total cardiac output at rest, which falls to approximately 3% of the (highly increased) cardiac output during heavy exercise. Blood flow, for example aortic blood flow, can be determined using echo Doppler or other techniques known in the art.

In a further embodiment of the invention, the method comprises the steps of making a surface abrasion on or near a superior lobe of a liver of the recipient and implanting the metanephros on or near the abrasion to allow the metanephros to connect to an hepatic blood supply. The Experimental section below shows evidence for improved development of metanephroi thus implanted.

In a preferred embodiment, the metanephros has an intact renal capsule.

In another embodiment of the invention, at least two whole metanephroi, each with renal capsules intact, are implanted into the recipient.

5. The metanephros may be allogeneic to the recipient. Alternatively, the metanephros may be xenogeneic to the recipient.

The metanephros is preferably derived from a non-human embryonic mammalian donor. Preferably, the non-human embryonic mammalian donor is porcine (for example a E26-E30 porcine embryo). Porcine embryos may be obtained from a pig or a herd of pigs known to be free of zoonoses. The herd of pigs should be free of porcine endogenous retroviruses, for example.

The mammalian recipient may human. Preferably, the human recipient is implanted with one or more porcine metanephroi.

- 20 The method may further comprise immunosuppressing the recipient, for example with an immunosuppressive regimen comprising an immunosuppressive agent or tolerance-inducing agent. Immunosuppression may take place prior to, during and/or after implantation or transplantation. Thus, a method of treating a subject suffering from kidney disease according to the present invention may comprise an additional step of treating the subject, prior to, during or following transplantation, with an immunosuppressive agent. This may include administration of an immunosuppressive agent and/or a graft donor-derived tolerance-inducing cell population which are known to those skilled in the art of kidney transplant immunology. Examples of immunosuppressive agents include, but are not limited to, CTLA4-Ig, anti-CD40 antibodies, anti-CD40 ligand antibodies, anti-B7 antibodies, rapamycin, (sirolimus), methyl prednisolone, azathioprine, cyclosporine A, cyclophosphamide and fludarabin. The immunosuppressive regimen is preferably not

nephrotoxic. Examples of tolerance-inducing cell populations include, but are not limited to, cells displaying a myeloid phenotype, and CD8.sup.+ T cells. The immunosuppressive regimen used with the method should preferably be approved by an appropriate regulatory body such as the FDA.

The metanephros may be obtained from the donor within certain stage after embryonic development of the metanephros begins. For example, the metanephros may be obtained from the donor within 2 to 4 days after embryonic development of the metanephros begins in rats, and a proportionate range if other species of donor are used. Table 1 shows the time-course in days of metanephros development and gestational period in some vertebrates.

Table 1

	<u>Metanephros</u> <u>Formation (days)</u>	<u>Gestational</u> <u>Period (days)</u>
Human	35-37	267
Macaque	38-39	167
Pig	20-30	114
Guinea Pig	23	67
Rabbit	14	32
Rat	12.5	22
Mouse	11	19
Hamster	10	16
Chick	6	21

The metanephros may be obtained from the donor prior to the presence of noticeable blood vessels (excluding early capillaries) within the metanephros.

The recipient may have reduced functional renal mass prior to implantation of the metanephros.

In a further embodiment, after the ureter of the chimeric kidney develops, a ureter to ureter anastomosis may be performed to provide fluid communication between the ureter of the chimeric kidney and a ureter of the recipient.

In another embodiment, after the ureter of the chimeric kidney develops, a ureter to bladder anastomosis is performed to provide fluid communication between the ureter of the chimeric kidney and the bladder of the recipient. In this embodiment, the metanephros is implanted preferably on or near the iliac vein or iliac artery.

In a further embodiment, the recipient's ureter is connected (for example, anastomosed) to a cyst (for example, containing urine) located around the ureter developed by the chimeric kidney. The cyst may be considerably larger and more visible than the chimeric kidney (graft) ureter and so allow easier connection to the recipient's ureter. In our experiments (below) we have shown that it is possible to anastomose the recipient's ureter to the wall of the cyst rather than to the chimeric kidney ureter per se.

According to a further aspect of the invention, two or more metanephroi are implanted into the recipient. The two or more metanephroi may be linked using an interconnecting manifold. The method of the invention includes forming a chimeric kidney multiplex as described above.

The mammalian recipient may be a juvenile or adult.

The metanephros may be implanted within five hours, preferably 2 to 4 hours, after removal from the embryonic donor.

Prior to implantation of the metanephros, renal tissue may be removed from the mammalian recipient.

In a further aspect of the invention the metanephros is transplanted to a site and connected to a large blood vessel via a tube or canula. Alternatively, angioplasty could be used to widen a smaller blood vessel to which the metanephros is connected. In this way, a smaller blood vessel is in effect engineered to become a large blood vessel as defined herein.

10 In a further aspect of the invention, there is provided a chimeric kidney obtainable using the method as described herein.

In another aspect of the invention, there is provided a method of growing a donor embryonic mammalian metanephros in a mammalian recipient, comprising the step of implanting the metanephros on or near a large blood vessel of the recipient under conditions that allow the metanephros to become vascularised. Donor embryonic mammalian metanephroi obtainable using this method are also covered by the present invention.

The invention further covers chimeric kidney tissue capable of producing urine and produced by the growth within a recipient after transplantation of one or more mammalian embryonic metanephroi at a site close to a large blood vessel within the abdominal cavity. The chimeric kidney tissue may be derived from renal mesenchyme and ureteric bud originating from one or more mammalian donor transplanted embryonic metanephroi (which for example do not have glomeruli, and do not contain arteries, arterioles, veins or venules at the time of transplantation) and a blood supply mostly derived from the recipient.

35 At about twenty days after transplantation, transplanted metanephroi of the invention may contain one or more of the following components:

(1) nephrons with glomeruli, for example between 500 and 2000 glomeruli, preferable more than 1000 glomeruli. The glomeruli may have an average diameter between 40 and 100um;

5

(2) a collecting duct system which forms an interconnecting manifold with the graft ureter and which can drains urine from the nephrons;

10

(3) a blood supply comprising arterioles, arteries, veins or venules as well as capillaries within the renal pelvis, and which enters the chimeric kidney through its hilum;

and/or

15

(4) a blood supply which is supplied by vessels originating from one predominant direction within the abdominal cavity of the patient.

20

The chimeric kidney of the present invention is capable of producing urine into the recipient's ureter and/or bladder. The ureter of the chimeric kidney may be anastomosed to the host ureter to form an interconnecting manifold for the excretion of urine via an end to end or end to side

25

anastomosis.

The chimeric kidney may be formed by anastomoses of the recipient's or host's ureter to the wall of a urinary cyst formed on the metanephros so as to form an interconnecting

30

manifold for the excretion of urine.

In a further aspect of the invention, there is provided a method of reducing the inflammatory or immune response following implantation of a donor embryonic mammalian

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metanephros in a mammalian recipient, comprising the step of implanting the metanephros on or near a large blood vessel

of the recipient under conditions that allow the metanephros to become vascularised.

The invention encompasses transplantation of metanephros under the membrane covering the aorta in humans, rats or other mammals or under the membrane covering either the renal or femoral artery in large mammals. This would allow the transplants to develop a blood supply from high pressure arterial sources.

The method of the invention may be extended to the formation of other chimeric organs, including pancreas and thymus, which requires good vasculature and appropriate rapid development.

Further aspects of the invention, including various embodiments by way of example, will now be described with reference to the drawings in which:

Fig. 1A,B are photographs showing differences in gross morphology between E15 metanephroi transplanted onto either the omentum (A) or near the renal vein (B) 16 days post transplantation in outbred Sprague-Dawley donors to outbred recipients. In (B), the arrowhead shows the venous return;

Fig. 2A-C are micrographs showing (A) rudimentary structures of metanephroi transplanted from E15 embryos outbred Sprague-Dawley rat donors into a host outbred Sprague-Dawley rat recipient, and development of mature structures in transplants 16 days post-transplantation from the omentum (B) and near the renal vein (C). G denotes a glomerulus and arrowheads denote nephron tubules;

Fig. 3A,B show lectin stained architecture of the collecting tubule system 35 days post-transplantation (from outbred Sprague-Dawley rat donors to outbred recipients) onto the omentum (A, top - photograph; bottom - micrograph)

and near the renal vein (B, top - photograph; bottom - micrograph). Arrows in Fig. 3A (bottom) show sites of inflammation. Arrows in Fig. 3B (bottom) show tubules and G denote a glomerulus;

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Fig. 4A,B show surface abrasion of the liver results in omentum metanephroi transplants (from outbred Sprague-Dawley rat donors to outbred recipients) with an hepatic blood supply (photograph A, arrowhead denotes blood vessel), and the development of mature kidney structures within 12 days of transplantation (micrograph B) including glomeruli (G), collecting tubules (CT), blood vessels (BV) and nephron tubules (arrowheads);

10

Fig. 5A,B show the development of metanephroi transplanted to the renal vessels (E15 Lewis donors to Lewis recipients) in the presence or absence (unilateral nephrectomy) of native renal mass. (A) is a diagrammatic representation showing venous blood vessels present after unilateral nephrectomy which surround and support the transplanted metanephroi. VC = vena cava, RV = renal vein, GV = gonadal vein, X = transplant location. (B) is a photograph showing metanephroi transplanted to the right renal vessels (next to native kidney) and left renal vessels (after unilateral nephrectomy), which both develop well and have urine cysts. The transplant to the left renal vessels has been connected to the host ureter via an end-to-end anastomosis. Left and right refer to the rat recipient (hence reversed in figure). RT = right transplant, CLT = connected left transplant, A = anastomosis, HU = host ureter, UC = urine cyst, NC = native kidney;

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Fig. 6A,B are histological sections to show the well developed glomeruli, tubules and collecting duct system in the metanephroi transplanted to the renal vessels 18 days after transplantation (E15 embryonic metanephroi from Lewis rat donor to Lewis recipient). (A) is a micrograph of the

medullar region of the transplant showing the numerous glomeruli (G, only some of glomeruli labelled) and tubules (P= proximal tubule, D = distal tubule). (B) is a micrograph of the cortical region of the transplant showing the well developed collecting duct (CD) system. Only a few of the collecting ducts are labelled for simplicity;

Fig. 7A-E show differences in development between metanephroi extracted from the same foetus and transplanted to the renal vessels or omentum (E15 Lewis donor to Lewis recipient). (A) and (B) show metanephroi transplanted to the renal vessels (A) and omentum (B) 18 days post-transplantation, the transplant to the renal vessels is larger (6.34mm long axis) than the transplant to the omentum (4.81mm long axis). The transplant to the renal vessels also contains a urine cyst (absent in the transplant to the omentum) which is aligned with the host ureter, making subsequent ureteroureterostomy simpler. In (A), HU = host ureter, UC = urine cyst. In (C), the renal vessels transplant (R) and omentum transplant (O) explanted from the animal to show differences in size. (D) shows an enlargement of the pelvi-calyceal region in (E) (see white rectangular box in (E)) with excellent delineation of collecting duct system. (E) and (F) are lectin stained whole mount transplants from the renal vessels (E) and omentum (F) showing the collecting duct system;

Fig. 8A,B show development of metanephroi transplanted onto the aorta. (A) is a photograph showing the metanephroi (Mt) placed upon the aorta (Ao). (B) is a histological section of the metanephroi transplanted onto the aorta 21 days post-transplantation, a large number of glomeruli (G, not all glomeruli labelled) have developed as well as the nephron tubules;

Fig. 9A,B show development of metanephroi transplanted onto the iliac vessels. (A) is a photograph of a metanephros

transplant (Tx) placed upon the iliac vessels 21 days post-transplantation. IV = iliac vein, HU = host ureter. (B) is a histological section of the metanephros transplanted onto the iliac vessels 21 days post-transplantation. A large number of glomeruli (G, not all glomeruli labelled) have developed in addition to the nephron tubules;

Fig. 10A,B show multiple transplants anastomosed to the host ureter 21 days after metanephroi transplantation. In (A), two transplants are aligned along the host ureter, with one transplant anastomosed end-to-end (E-E) and one transplant anastomosed end-to-side (E-S). (B) is an overlay of picture A, showing the host ureter in dark grey, the two transplants highlighted by white dashes and the points of anastomosis shown by X;

Fig. 11A-D show a method for joining two transplant ureters to the host ureter using only end-to-end anastomosis. This was performed on E15 metanephroi transplanted from inbred Lewis donors to inbred Lewis recipients. (A) is a photograph demonstrating two transplants (Tx1 and Tx2) which were transplanted in apposition with their ureters in close proximity to each other. (B) is a photograph of both transplant ureters (Tx ureter 1 and Tx ureter 2) anastomosed end-to-end with the host ureter. (C) and (D) are diagrams illustrating the method of joining both transplant ureters (C ureters 1 and 2) with the host ureter. Numbers in C and D relate to the stitches cast during the anastomoses. In (A)-(C), U = ureter, HU = host ureter, A = anastomosis;

Fig. 12 shows histology of transplants to the fascia of the psoas muscle. Transplants to this region develop both glomeruli (G) and proximal and distal tubules (T);

Fig. 13A,B are diagrams showing (A) an end to end anastomosis of the graft ureter of the chimeric kidney to

the recipient ureter. The anastomosis can be performed after the urinary cyst around the graft ureter has been carefully opened; and (B) anastomosis of the recipient ureter directly to the wall of the urinary cyst which surrounds the graft ureter. Urine will be released through the graft ureter into the urinary cyst but can only be released through the recipient ureter;

Fig. 14A-D illustrate how the blood supply enters the chimeric kidney through the hilum in various situations. In (A), a native kidney (not to scale) in which the renal artery divides into three near the hilum is shown. The renal artery is a primary branch of the aorta. (B) shows how the omentum which is fed by numerous small vessels throughout the omentum all of which are branches of the gastro-epiploic artery, itself a branch of the lineal branch of the caeliac artery itself a branch of the aorta. (C) shows how with renal capsule transplantation blood vessels are not limited to entering the chimeric kidney through the hilum. (D) shows a renal vein site as an example of sites of implantation suggested by the present invention which most closely resembles the blood supply of the native kidney; and

Fig. 15 is a diagram showing a frustoconical section of an area deriving blood vessels which enter through the hilum.

Experimental

Locations within the peritoneal cavity have been investigated as sites for metanephroi transplantation.

Initial experiments were performed on out-bred Sprague-Dawley rats without immunosuppression. These experiments demonstrate both developmental and immunological advantages of the new surgical sites compared with the omentum. As rejection occurred in most of the outbred omentum transplants, it is noted that assessment of developmental and morphological differences in the resultant chimeric kidneys in the absence of immunosuppression is not clear.

Later experiments were performed on inbred Lewis rats without immunosuppression to demonstrate the pure developmental and morphological differences of chimeric kidneys born at different surgical sites in the absence of a significant immune response.

Example 1: Transplantation of metanephroi to the renal vein from Sprague-Dawley donors to Sprague-Dawley recipients

Materials & Methods

Extraction of metanephroi

A growth factor cocktail was prepared at the following concentrations: Human recombinant IGF-I 10^{-7} M (Upstate Biotech #01-208) Human recombinant IGF-II 10^{-7} M (Upstate Biotech #01-142), Human recombinant TGF α 10^{-8} M (Upstate Biotech #01-165), Human recombinant HGF 10^{-8} M (R&D systems Ltd. #294-HGN-005), Human recombinant VEGF 10^{-7} M (Upstate Biotech #01-185), Human recombinant FGF 5ug/ml (R&D systems #234-FSE-025), Human recombinant NGF-I 5ug/ml (R&D systems #256-GF-100), Retinoic acid 10^{-6} M (#R2625), Corticotropin

releasing factor 1µg/ml (Sigma #C3042), Iron saturated transferrin 5µg/ml (Sigma #T8158).

Embryonic day 15 metanephroi were extracted from time-mated Sprague-Dawley rat embryos using a dissecting microscope. Only metanephroi that contained a reasonable length (approximate minimum of 0.1mm) of ureter were selected for transplantation. The metanephroi to be transplanted were placed in the growth factor cocktail outlined above, for between 2 to 5 hours on ice prior to transplantation.

Surgery

250g female Sprague-Dawley rats were anaesthetised by intraperitoneal injection of ketamine (90mg/Kg) and Xylazine (16mg/Kg). Once sufficient anaesthesia had been achieved the animal was immobilised and the abdomen was shaved and swabbed with antimicrobial solution. A midline incision through the skin sufficient in length for subsequent procedures (approximately 4cm) was created, this incision was then deepened through the linea alba and peritoneum to open the peritoneal cavity and was kept open using a self retaining retractor. The mobile viscera was gently lifted to the right side of the abdomen and covered with a moist swab to prevent drying. A pouch was created in the retroperitoneal fat close to the renal vein near the junction with the gonadal vein using forceps and a single metanephroi (pre-incubated in the growth factor cocktail) was gently placed into the pouch, which was then closed using 8/0 chromic cat gut suture (Ethicon). The viscera was gently replaced back into the peritoneum and the laparotomy was closed in two layers using 3/0 vicryl suture (Ethicon) and the animal was recovered.

Analysis of metanephroi

Sixteen days after transplantation, the rat was euthanased by dislocation of the neck, the laparotomy was re-opened and the transplanted metanephroi identified and removed for

analysis.

Renal histology

Tissue samples were fixed in 2% paraformaldehyde overnight at 4°C, then dehydrated and embedded in paraffin. Five micrometer sections were cut and mounted and stained with Harris's haematoxylin and eosin prior to being viewed by light microscopy.

Lectin staining (Dolichos biflorus (DB) lectin)

Tissues were fixed in 2% paraformaldehyde for 30 minutes at 4°C, permeabilised with 0.1% saponin and then incubated with FITC-conjugated DB (50µg/ml, Vector Labs.) in a humidified chamber for 60 minutes at 37°C. After extensive washing, tissues were post-fixed in 2% paraformaldehyde again for 5 minutes and viewed by standard immunofluorescence microscopy. The use of DB lectin to demonstrate functional maturation of the nephron has been described previously (Laitinen L. et al., 1987, J. Histochem. Cytochem. 35: 55-65).

Results

Metanephroi transplantation near the renal vein can result in the transplants deriving a blood supply from either the renal vein or gonadal vein (both of which carry large volumes of blood). Transplants growing near the renal blood supply sometimes appear to have a better gross morphology i.e. they more closely resemble the native kidney in terms of shape and colour compared to samples transplanted onto the omentum as shown in Fig. 1.

Light microscopy reveals that samples grown on the omentum and near the renal vein both develop mature glomeruli and undergo tubulogenesis to form the distal and convoluted tubules of the mature nephron within 12 to 16 days post-

transplantation (see Fig. 2).

However, the transplants near the renal vein develop with more tubules and more glomeruli. Moreover, these glomeruli are larger in size.

Lectin staining reveals the superior collecting duct system of the chimeric kidneys derived from metanephroi transplanted near the renal vein compared with those transplanted near the omentum.

Fig. 3 shows lectin staining to show the architecture of the collecting tubule system 35 days post-transplantation that transplants onto the omentum develops a sparse collecting system with no defined symmetry (Fig. 3A top), further more, histologically the transplants onto the omentum have large areas of inflammation and lack either defined glomeruli or tubules (Fig. 3A bottom). This may be due to rejection of the transplanted metanephroi and a significant lymphocyte infiltrate is visible (see Fig 3A bottom). By comparison the transplants near the renal vein have a well-developed collecting system which displays both symmetry and the presence of adult structures such as the kidney medulla (Fig. 3B top). Histologically these transplants have mature glomeruli which are extremely abundant (Fig. 3B bottom). Moreover, the lymphocyte infiltrate is less severe. One could therefore predict that metanephroi transplanted near to the renal vein would develop into more functional chimeric kidneys than those transplanted to the omentum.

Discussion

Advantages of this new site(s) of transplantation (close to renal vein) from the above-described experiments in outbred Sprague-Dawley rats include:

- Improved development of kidney in terms of number, size

and maturity of glomeruli

- Improved collecting duct system
- Improved blood pressure supply to the transplants which may improve function
- 5 • Improved location close to native renal tissue, which may improve further procedures such as anastomosis of ureters
- Improved development of transplants in the absence of either unilateral or 5/6 contralateral nephrectomy
- 10 • Reduced inflammation and/or immune response.

The amount of function Hammerman has observed when transplanting metanephroi into adult hosts may not entirely be related to their innate functional ability. For example,
15 the low renal function (typically 3%, maximum 5-7%) of the metanephroi may be related to the blood flow into the omentum. It is currently thought that higher blood pressure provided at the sites mentioned in the present invention may improve chimeric kidney function.

20 The reduction in inflammatory and/or immune response which is clear in above data for the transplant of metanephroi near the renal vein compared with controls of the omentum may be counter-intuitive. One might expect that there would
25 be a greater number of white blood cells in the vicinity and potentially able to enter the graft when transplanted into a site which will be better vascularised. However, the extravasation of leukocytes and overall blood flow do not necessarily correlate since it is an active not a passive
30 process.

Example 2: Surface abrasion of liver in transplantation
from Sprague-Dawley donors to Sprague Dawley recipients

35 A novel alternative method for increasing blood flow to metanephroi is to create a surface abrasion on the superior

lobe (right lobe) of the liver.

Materials & Methods

5 Extraction of metanephroi

The extraction of the metanephroi from E15 time mated Sprague-Dawley rats and the pre-transplantation incubation in growth factor cocktail was the same as Example 1.

10 Surgery

250g female Sprague-Dawley rats were anaesthetised by intra-peritoneal injection of ketamine (90mg/Kg) and Xylazine (16mg/Kg). Once sufficient anaesthesia had been achieved the animal was immobilised and the abdomen was shaved and
15 swabbed with antimicrobial solution. A midline incision through the skin sufficient in length for subsequent procedures (approximately 3cm) was created, this incision was then deepened through the linea alba and peritoneum to open the peritoneal cavity and was kept open using a self
20 retaining retractor. The omentum was spread out using sterile cotton buds and a pouch was created through the surface membrane. A single metanephroi (incubated in growth factors) was then places into the pouch. A surface abrasion of approximately 1mm was then made onto the superior lobe of
25 the liver using micro-dissection scissors. The laparotomy was closed in two layers using 3/0 vicryl suture and the animal was recovered.

Results

30

Metanephroi transplanted into a pouch on the omentum in close proximity to a surface abrasion of the superior lobe of the liver can cause vasculature outgrowth from the liver to the metanephroi and allows the transplant to connect to
35 the hepatic blood supply (Fig. 4A). Histologically these transplants have developed mature structures within 12 days of transplantation (Fig. 4B).

Example 3: Alternative surgical locations

5. An alternative location for metanephroi transplantation is close to the iliac blood supply. This is the site of choice for current human whole organ kidney transplants due to the blood volume and pressure of this system. Furthermore, transplantation of metanephroi into this region may allow
10 anastomosis of the transplant ureter to the host bladder using an antirefluxing extravesical ureteroneocystostomy to prevent reflux of urine from the bladder into the metanephroi transplant. Preliminary experiments have shown that metanephroi transplanted into this region do develop.

15

Example 4: Metanephroi transplants from Lewis inbred donors to the renal vessels of Lewis inbred recipients

- 20 These experiments were performed on inbred animals so as to reduce the influence of any immune rejection on the development of the transplanted metanephroi.

Materials & Methods

25

Extraction of metanephroi

- The growth factor cocktail used for pre-transplantation incubation of the metanephroi was the same as for Example 1, with the addition of Prostoglandin E1 $7 \times 10^{-11} \text{M}$ (Sigma #P5640)
30 and Tamm-Horsfall protein 1ug/ml (Biomedical Technologies #BT-381). The extraction of metanephroi was as described in Example 1, except that time-mated Lewis inbred rats were used.

Surgery

35

200g female Lewis inbred rats were anaesthetised by continual administration of isoflurane gaseous anaesthesia.

Once sufficient anaesthesia had been achieved the animal was immobilised on a heating mat and the abdomen was shaved and swabbed with antimicrobial solution. A midline incision through the skin sufficient in length for subsequent procedures (approximately 3cm) was created, this incision was then deepened through the linea alba and peritoneum to open the peritoneal cavity and was kept open using a self retaining retractor. The mobile viscera of the peritoneal cavity was gently lifted to right side of the abdomen to reveal the left kidney and covered with a moist swab to prevent drying. Next the renal vein, renal artery and ureter of the left kidney were isolated through sharp and blunt dissection and ligated using 5/0 vicryl suture (Ethicon). A small incision was made in the renal capsule of the left kidney which was then extended to perform a sub-capsular nephrectomy. Once the kidney was removed a small pouch was created in the retro-peritoneal fat close to the point of ligation of the renal vessels and a single metanephroi soaked in the growth factor cocktail outlined above was placed into the pouch. The viscera was gently replaced back into the peritoneum and the laparotomy was closed in two layers using 3/0 vicryl suture (Ethicon) and the animal was recovered.

Inulin Clearance

The function of the transplanted kidneys can be assessed by standard techniques such as inulin clearance. The ability of the transplanted metanephroi to perform their function as kidneys is measured through their capacity to remove inulin from the blood, a measure of the transplants glomerular filtration rate.

To measure inulin clearance, the abdomen is opened through a midline laparotomy and a contra-lateral subcapsular nephrectomy is performed on the right kidney in order to remove all remaining native renal mass, the left kidney having been removed at the time of transplantation.

Meanwhile, a portion of the bladder is mobilised and a hole is created using a diathermy unit, a catheter is then placed into the bladder through the hole and sealed using suture. The laparotomy is closed to prevent drying of the viscera. Next a midline incision is made into the neck, typically extending from the clavicle to just below the lower mandible. The sternothyroideus muscle is then separated from the sternomastoideus using blunt dissection to reveal the jugular vein and carotid artery. A section of each vessel is mobilised using forceps and tied at the rostral end to prevent blood flow, catheters primed with saline are then placed into the jugular vein to continually infuse the inulin and into the carotid artery to monitor the physiological parameters of the animal.

The inulin clearance is then determined by infusing tritiated (^3H) inulin into the animal, until a steady state blood concentration is achieved. Using the FICK principle the rate of elimination of inulin from the blood (into the urine) can be deduced using the following equation:

$$\text{Clearance of Inulin} = \text{Urine Flow Rate} \frac{[\text{urine inulin concentration}]}{[\text{plasma inulin concentration}]}$$

where urine flow rate is measured by weight and the concentration of inulin in the urine and plasma are expressed as scintillation per minute of 10ml aliquots in 4ml of Optiphase HiSafe 2 scintillant measured on a beta counter. Given that inulin is freely filtered at the glomerulus and neither secreted nor resorbed in the tubules, this provides a measure of the glomerular filtration rate.

Results

Metanephroi were transplanted near the left renal vessels

which have been ligated during the unilateral nephrectomy step (Fig. 5). They appear to have had the ability to derive a blood supply from the renal vasculature. The transplants also underwent growth and development which is indistinguishable from transplants performed near intact renal vessels that have not undergone ligation (Fig. 5B).

The point of ligation during the unilateral nephrectomy step is such that the gonadal vein remained intact with a venous return which flowed into the renal vein (Fig. 5A). In a number of cases the transplants appeared to derive their venous blood supply from the gonadal vein, while in other cases, the venous return was directly into the renal vein through new vessels running from the transplant into the renal vein.

In the majority of cases, a urine cyst was found at the hilum of the transplants near the renal vessels (Fig. 5B). This cyst can be used to identify and orientate the hilum. Careful dissection into this cyst revealed the transplant ureter.

Transplantation of metanephroi near the renal vessels resulted in transplants that were in an ideal location for connection to the host ureter, and in many cases the transplant overlays the host ureter which would give the potential to connect multiple transplants to a single ureter.

Light microscopy revealed that transplants near the renal vessels developed numerous mature glomeruli (see Fig. 6A) and tubules including both proximal and distal tubules as well as a collecting duct system (see Fig. 6B) with a defined medullar region. There was also no sign of rejection in the inbred transplants.

Example 5: Paired metanephric transplants from Lewis donors to renal vein and omentum in inbred Lewis recipients

Materials & Methods

5 The pre-transplantation growth factor cocktail was the same as that described in Example 4. Pairs of embryonic day 15 metanephroi were extracted from time mated Lewis inbred rat
10 fetuses, and kept together for transplantation into the same recipient animal. All paired metanephroi were incubated in the growth factor cocktail outlined above for between 1 and 3 hours on ice prior to transplantation. Lectin staining was as described in Example 1. Samples for lectin staining were dissected in half along their long axis prior to
15 commencement of the staining procedure.

Surgery

The surgical procedure was the same as outlined in Example 4, with the exception that one of the paired metanephroi was
20 placed into a pouch near the renal vessels, then once the viscera was replaced into the peritoneum the omentum was spread out using sterile cotton buds and a pouch was created through the surface membrane and the other paired metanephroi was placed into the pouch. The laparotomy was
25 then closed in two layers using 3/0 vicryl suture (Ethicon) and the animal was recovered.

Results

30 Using paired metanephroi from the same foetus reduced experimental variation allowing differences in location on transplant development to be examined unambiguously. Upon initial examination of the rats 18-21 days post transplantation, it was observed that both transplants had
35 grown and appeared to be healthy (Fig. 7A, B). Further examination revealed that the renal transplants had developed a urinary cyst (Fig. 7A), which was absent on the

omentum transplants. This suggests better development of the transplants at the renal site. Removal of the transplants from the animal showed that the renal transplant was larger in size than the omentum transplant (24% larger along its long axis than the transplant onto the omentum; see Fig. 7C).

Lectin staining of the paired transplants showed that the transplant to the renal vessels has a much better developed collecting duct system (compare Fig. 7E with Fig. 7F). It can be seen that the transplant to the renal vessels has an increased development of the pelvi-calyceal system with better delineation of the collecting ducts (Figs 7D and 7E)

Example 6: Metanephric transplants from Lewis donors to Aorta in inbred Lewis recipients

Materials & Methods

The growth factor cocktail and metanephroi extraction technique was as described in Example 4. The surgical procedure was as described in Example 4 with the exception that instead of transplanting metanephroi into a pouch near the ligated renal vessels, the metanephroi were transplanted into pouches created above the aorta.

Results

Metanephroi transplanted to a retro-peritoneal pouch above the aorta, resulted in transplants which derived their blood supply from the aorta and vena cava. Vascular development was apparently achieved via budding of new vessels directly from the aorta and vena cava (see Fig. 8A). The metanephroi developed and histological analysis of sections revealed significant numbers of glomeruli were present (see Fig. 8B). Because the aorta and vena cava are major blood vessels its

might be expected that transplant development would be improved, it was therefore surprising that histological analysis revealed a lower degree of glomerularogenesis in transplants to the aorta and a less healthy appearance of the transplants at the structural level, with less well defined tubular structures (see Fig. 8b). However preliminary analysis did suggest a high proportion of transplanted metanephroi to the aorta had urinary cysts around their ureter.

Example 7: Metanephric transplants from Lewis donors to iliac artery in inbred Lewis recipients

Materials & Methods

The growth factor cocktail and metanephroi extraction technique was as described in Example 4. The surgical procedure was as described in Example 4 with the exception that instead of transplanting metanephroi into a pouch near the ligated renal vessels, the metanephroi were transplanted into a pouch created above the iliac vessels.

Results

Metanephroi transplanted near the iliac vessels results in transplant which derived their blood supply directly from the iliac system. The gross morphological appearance of metanephroi transplanted to the iliac vessels (21 days after transplantation) was good (see Fig. 9A), and the transplants appeared to be well vascularised and healthy. In a similar manner to metanephroi transplanted near the renal vessels, the iliac transplants were in an ideal location for connection to the host ureter and in many case actually rested upon the host ureter (see Fig. 9).

However, in this instance light microscopy revealed,

surprisingly, that although glomerulogenesis has occurred, the number of glomeruli is lower than seen in transplants to the renal vessels (see Fig. 9B and compare with Fig. 6 for renal vein site). The transplants also lack clearly
5 developed tubules and collecting ducts and look generally less healthy than transplants to the renal vessels.

Therefore one may conclude that although proximity to the iliac artery is where existing allograft kidney
10 transplantations are performed, and could be a suitable site for metanephric transplantation. However, transplantation on or near the iliac artery may be a less suitable embodiment than proximity to the renal vein or artery.

15

Example 8: Transplantation of metanephroi to the renal vessels and connection of multiple transplants to the host ureter

20

Materials & Methods

The growth factor cocktail and metanephroi extraction technique was as described in Example 4. The surgical procedure was as described in Example 4 with the exception
25 that multiple metanephroi were transplanted into pouches near the ligated renal vessels.

Results

30

Transplantation of multiple metanephroi to the renal vessels resulted in transplants aligned with the host ureter. Careful liberation of the ureters of the transplants allowed both end-to-end and end-to-side anastomoses (Fig. 10 A and B). Anastomosis of more than one transplant with the host
35 ureter should give better combined renal function over a single transplant alone. This cannot not be performed with transplants to the omentum due to the tension created during

the initial end-to-end anastomosis (longer distance for the host ureter to stretch to the omentum) which results in insufficient host ureter length availability for subsequent transplant connections.

5

Transplantation of two metanephroi in apposition resulted in transplants with ureters in close proximity to each other (Fig. 11 A). If the distance between the ureter was sufficiently low, then a double end-to-end anastomosis could be performed with the host ureter. First, both transplant ureters were carefully liberated and joined though two stitches on the inner surfaces (Fig. 11C stitches 1 and 2), next the outer sides of the transplant ureters were anastomosed with the host ureter through 2 more stitches (Fig. 11D, stitches 3 and 4), and finally two more stitches were cast at the front and back of the host ureter (Fig. 11D stitch 5) creating a patent anastomosis between all 3 ureters. Joining two transplant ureters in this manner could improve the combined transplant renal function when compared with a single transplant anastomosis.

20

Example 9: Comparative quantitative analysis of transplantation of embryonic metanephroi from Lewis rat donors into Lewis recipients at different implantation sites

25

Material & Methods

To assess the relative potential advantages of the new sites within the abdominal cavity a retrospective study of the data described in the above examples was performed. All experiments were performed using E15 embryonic metanephroi obtained from time-mated Lewis rats donors and transplanted without immunosuppression into inbred Lewis rat recipients.

35

Omentum transplants, renal vein transplants and iliac vein transplants were performed as described above.

Transplantation of embryonic metanephroi to the psoas fascia was performed as described below. The psoas represents a site not immediately proximal to a large blood vessel, and serves as a "negative control". It was intended to identify whether "any site within the abdomen" could suffice for successful embryonic metanephric transplantation. The growth factor cocktail and metanephroi extraction technique was as described in Example 4. The surgical procedure was as described in Example 4 with the exception that instead of transplanting metanephroi into a pouch near the ligated renal vessels, the metanephroi were transplanted into pouches created in the fascia of the psoas muscle.

The various sites were compared by assessment of the metanephric transplants for the following parameters:

(1) Growth, as assessed by both size and morphological appearance: only a proportion of metanephroi will develop from an E15 metanephros to form a "chimeric kidney";

(2) Possible connectivity of the transplant ureter. A ureter will only develop in certain transplants, and connectivity to the host's ureter can be determined; and

(3) The presence or absence of visible cysts or sacs containing urine (referred to below as urine cysts) on the surface of the chimeric kidney, before connection of the transplant ureter to the recipient ureter. This represents a "surrogate endpoint" as an alternative to inulin clearance measurements. The surrogate endpoint may be viewed as an approximation of whether the resulting chimeric kidney will be essentially functional and capable of producing urine. It will be determined by the presence of a functional collecting duct system within the chimeric kidney. However, it is possible that a chimeric kidney could be functional after ureter connection has been performed, without the

presence of a urine cyst before connection. Therefore, the endpoint is not definitive and endpoint figure may be an underestimate of the likely success of a procedure at producing a chimeric kidney. Furthermore, some transplants contain urine cysts which were not macroscopically visible either due to their small size or because they were obscured (e.g. underneath the transplant).

Statistical analysis on data in Tables 2-4 was performed using either chi square analysis, unpaired T-tests or Mann-Whitney analysis as appropriate.

Results and Discussion

Transplantation of metanephroi onto the fascia of the psoas muscle (the muscle responsible for flexing the thigh upon the pelvis) is an example of a transplantation site which is supplied by the iliac artery, but not directly off the systemic blood flow. Rather, the fascia blood flow is off a microvascular bed derived from the iliac artery in a manner similar to the omentum blood flow which is derived from the gastro-epiploic artery. In the omentum, the left gastro-epiploic artery is a branch from the lienal artery, a branch of the caeliac artery, which is itself a branch of the aorta. The left gastro-epiploic artery which anastomoses with the right gastro-epiploic artery is a branch of the hepatic artery, which is a branch of the caeliac artery and of the aorta.

Metanephroi transplanted to the psoas fascia undergo both glomerulogenesis and tubulogenesis to form mature kidney structures (see Fig. 12). However, the transplants to the psoas fascia are smaller than metanephroi transplanted to the renal vessels and omentum (usually 1/3 the size) and have a lower glomerular density after an average of 20 days post-transplantation. Furthermore, fewer of the transplants to the psoas fascia developed after transplantation compared

to the renal vessels although those that did grow developed urine cysts indicating urine production and kidney function.

5 Table 2 illustrates areas in which transplantation of kidney metanephroi at or near the renal vein and to a lesser extent the aorta are superior to the omentum or psoas muscle.

10 First, a metanephros is more likely to grow at the renal site than at the omentum.

Second, once the metanephros grows it also is more likely to develop a connectable ureter if implanted at the renal vein site (or the aorta) than on the omentum.

15 Third, an implanted metanephros is more likely to have urine-containing cysts on its surface at the renal vein or aorta than the omentum. This suggests that metanephroi implanted at or near a large blood vessel is more likely to successfully develop a functional collecting duct system.

20 The difference in apparent growth potential of the various sites is shown in Table 2. The data in Table 2 demonstrates that when one compares the renal vein with implantation on the omentum in inbred Lewis rats, a higher proportion of metanephroi grow (76.5% vs 63%). This may be attributable to induction of a superior blood supply (see General Discussion below).

25 However, if one then classifies the metanephroi that grow successfully into those that have a visible ureter and are therefore "connectable", further advantages of the renal vein site compared with the omentum become apparent (see Table 2 - 59.6% vs 27.5% connectable). In this instance, the aorta demonstrates the same advantages as the renal vein site compared with the omentum.

35 At the renal vein and aorta sites, a similar proportion of

growing metanephroi developed urine cysts, and these were at a higher proportion than for the omentum (38.5% for renal vein vs 13.7% for omentum). This data suggests different inductive potentials of the various surgical sites for implantation of embryonic metanephroi to develop a functional collecting duct system. This observation is borne out by histological analysis using *Dolichos biflorus* staining of the collecting duct system described in Example 5. Again, in this instance, the aorta also demonstrates higher values than the omentum.

Table 2

	Omentum	Renal Vein	Aorta	Psoas
Number of recipients	27	26	7	13
Number of metanephroi transplanted	81	68	24	33
Number of metanephroi grown	51	52 ^{ss}	9	14 [#]
% Metanephroi grown	63.0	76.5	37.5	42.4
Number of transplants that were connectable	14	31 ^{##}	4	8
% Transplants that were connectable	27.5	59.6	44.4	57.1
Number of animals with transplants connected	14	22	4	8
% Animals with transplants connected	51.9	84.6	57.1	61.5
Number of transplants with urine cyst	7	20 ^s	4 ^{**}	5
% Transplants with urine cyst	13.7	38.5	44.4	35.7

p=0.05 compared to renal

\$ p=0.004 compared to omentum

** p=0.03 compared to omentum

p=0.001 compared to omentum

\$\$ p=0.075 compared to omentum

5

It is noted that in these transplants using inbred animals, the advantages of transplanting metanephroi at or near a large blood vessel (for example, advantages in size of metanephroi and size, number and development of glomeruli) are not as apparent in certain respects as the differences using outbred animals described in Examples 1-3 above.

10

15

Table 3 illustrates that in inbred Lewis-Lewis transplants there are no apparent differences between the mass or linear dimensions of chimeric kidneys at the omentum compared with renal vein sites. Within these inbred animals, in contrast to the situation in the outbred Sprague-Dawley transplants (see Examples 1-3 above), there is no evidence that the renal vein site leads to larger metanephroi than the omentum. It is therefore possible that the differences observed in the Sprague-Dawley transplants described in Example 1 are attributable to "immunological differences" between the sites, e.g. increased rejection of allogeneic transplanted metanephroi of outbred origin in the omentum compared with sites of the present invention.

20

25

30

In a preferred embodiment where porcine metanephroi are used from implantation into human patients, there are potential immunological advantages of the sites of the present invention, particularly the renal vein.

35

In the negative control of the psoas fascia, transplanted metanephroi are smaller than either the new sites or the omentum.

Table 3

	Omentum	Renal Vein	Psoas
Number of Recipients	11	10	4
Number metanephroi measured	18	13	6
Average mass	68 ± 67	50 ± 29	21 ± 11
Average length	5.81 ± 2.16	6.03 ± 1.21	4.33 ± 0.96
Average width	4.63 ± 1.63	4.47 ± 0.97	3.19 ± 0.69
Average pseudo volume*	101.7 ± 106.4	84.2 ± 45.0 ^s	31.2 ± 22.0
Days post transplant	19.8 ± 4.1	19.8 ± 1.0	20.0 ± 1.1

5 *calculated from the volume of a sphere using half the
average of the width and the length as the radius.

^s p=0.01 compared to psoas

10 Table 4 compares a preferred embodiment of the new surgical
sites, i.e. the renal vein, with the omentum and the psoas
fascia in inbred Lewis-Lewis transplants. In terms of
glomerular density and size, the metanephroi implanted at
the omentum and renal vein are similar. The psoas group also
15 had glomeruli with the same size and density, although
histological analysis suggested the glomeruli of psoas-
developed metanephroi were less healthy. It may be that
metanephroi are generally less sensitive to surgical site in
terms of their potential for developing glomeruli but differ
20 rather in their ability to form a functional collecting duct
system.

Table 4

	Omentum	Renal Vein	Psoas
Number of Recipients	7	7	2
Number metanephroi measured	8	10	4
Pseudo Glomerular total*	1,632 \pm 1,577	1,096 \pm 547	442 \pm 360 ^s
Glomerular Density	12.6 \pm 3.3	13.0 \pm 4.3	10.7 \pm 4.4
Glomerular Diameter	49.4 \pm 4.1	47.3 \pm 4.3	47.8 \pm 2.8

5

*calculated from pseudo volume (table 2) multiplied by density.

^s p<0.05 compared to omentum; p<0.05 compared to renal.

10

Example 10: Procedure for treatment of human patients with porcine metanephroi

15

In a preferred embodiment described below, one or more pig embryonic kidney metanephroi are transplanted into a human recipient.

Metanephros isolation

20

Sows (under appropriate rearing conditions, e.g. following Good Manufacturing Practice) are brought into oestrous either by natural means (housing near males) or more likely by hormone injections known to those skilled in the art.

Once the sows reach oestrous they are inseminated with semen

from tested boars of a suitable quality. Following insemination, sows are monitored and tested for pregnancy as appropriate. At 28 days of gestation (full gestation 112-115 days), sows are anaesthetised and a hysterectomy performed. The uterus is passed to a clean room and opened under sterile conditions (e.g. flow cabinet). Individual embryos are removed, and are held in cold buffer before dissection of the metanephroi. Once they have been dissected, metanephroi are stored in vials. Each vial is labelled such that metanephroi can be traced back to specific pig. Appropriate samples may be taken for biological safety evaluation. After biological safety clearance, vials are shipped to a clinical centre (for example, a hospital). Alternatively, product (i.e. metanephroi) isolation may take place the clinical centre, and final safety evaluation is then obtained retrospective (as for Diacrin's pig neural cell transplants for Parkinson's disease).

20 Metanephros Transplantation

Depending on the immunosuppressive protocol adopted (known to those skilled in the art and which may include one or more drugs such as cyclosporine, FK506 or antibodies such as CTLA4-Ig), the patient may receive immunosuppressive agents several days prior to receiving the porcine metanephric transplant.

While the patient is being prepped for surgery, the metanephroi are prepared for transplantation. Metanephroi are pre-treated for a time period (for example, 45 minutes) in a growth factor cocktail on ice as described in earlier Examples. The cocktail may contain (but is not limited to) one or more of the following growth factors: hepatocyte growth factor (HGF), insulin-like growth factor (IGF)-I, IGF-II, transforming growth factor- α (TGF α), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), nerve growth factor (NGF), retinoic acid,

corticotropin-releasing hormone, Tamm Horsfall protein, prostaglandin E1 and iron-saturated transferrin.

The patient's abdomen is opened, and one or more metanephroi transplanted to the preferred site. Between one and six metanephroi are transplanted. Preferably two to six metanephroi are transplanted to take into account possible transplant failure.

After the operation, the patient would probably stay in the clinic or hospital to recover (for example, for about 3 or 4 days). However, it is possible that transplantation may be performed using a laparoscope, and if so the recovery time would be much shorter.

Initially, the patient may continue to undergo their normal dialysis programme. The patient is monitored as required for the immunosuppressive regimen. The patient could return for imaging after a suitable time following discharge (for example, about 2-3 weeks following discharge). During the imaging procedure (e.g. Magnetic Resonance Imaging or Positron Emission Tomography), the degree of transplant development is assessed and this information used to schedule a second surgical step.

Urinary Connection

As the metanephros matures, it begins to produce urine. If urine is not allowed to flow (i.e. an obstruction occurs), the backpressure produced inhibits nephrogenesis, and inhibits kidney development. However, time is required for the ureter to reach a size that allows it to be anastomosed to the host urinary tract. Hence there is an optimum time, which is assessed clinically, to perform the second surgical step.

Prior to the second surgical step at the clinic or hospital, the surgeon plans the procedure based on the imaging data,

perhaps with further imaging taken earlier on the day or the day before. The patient's abdomen is opened again as appropriate to the transplant site. Developed metanephroi are mobilised, and one or more connected to the host's urinary system either by direct anastomosis to one of the host's ureters (end to side) or by the use of other tubing. Excess transplants may be removed at this stage. Further growth factors as above may be applied topically to the transplant at this stage. The patient is likely to require a stay in the clinic or hospital to recover, for example of four days or so.

For a period of time before urinary connection, and following connection until equilibrium is reached, the patient may be asked to collect total daily urine samples. The volume of the daily urine samples is monitored and samples tested. In addition, the patient may have daily blood samples taken to monitor electrolytes (in addition to whatever is needed to monitor immunosuppression). Based on clinical signs, dialysis may be continued as required, but slowly reduced (and eventually terminated) as metanephros function increases. Imaging is preferably performed regularly during the early follow-up period to monitor metanephros development and check for potency of ureter connections.

General Discussion

In experiments with inbred (i.e. allogeneic) animals, there are at least two unexpected advantages of implantation of embryonic metanephric tissue at or near a large blood vessel (for example, the renal vein) compared with prior art methods, i.e. a greater likelihood of the metanephroi having a connectable ureter, and a greater chance of developing urine-containing cysts.

In the experiments with outbred allogeneic animals, there were additional unexpected advantages for chimeric kidneys produced by implantation at or near a large blood vessel over the prior art methods: an improved chance of a
5 transplanted metanephroi growing, more glomeruli, larger glomeruli, and an larger overall size.

In both inbred and outbred animals, transplantation on or near the renal vein resulted in an improved chance of a
10 transplanted metanephroi growing compared with the omentum.

In chimeric kidneys from both inbred and outbred transplantations there were better collecting duct systems.

15 In all animals, because of closer proximity to the host ureter in for example the renal vein than in the omental site there is another advantage of the invention, i.e. easier connection of chimeric kidney to the recipients urogenital system either via connection of the graft ureter
20 to the recipient ureter via either an "end-to-end" anastomosis, "end-to-side" anastomosis or via direct anastomosis of the wall of a urine containing cyst to the recipient's ureter (see Fig. 13).

25 Furthermore, as metanephric typically have displayed 3% of normal renal function, one aspect of the invention (in all animals) is implantation of multiple metanephroi, allowing multiple connection to the host's ureter. Here, the new surgical sites have a significant advantage over the prior
30 art sites such as the omentum where a graft ureter is usually difficult to see and manipulate.

The method of the present invention result in higher levels of surgical success. This represents an economic advantage
35 to the new surgical sites. In one embodiment, where porcine embryonic metanephroi are used for implantation into human patients, the process for production of the embryonic

metanephroi in porcine donors in a controlled environment will not be inexpensive. Surgery will further represent a major cost. Therefore, improvement in the efficiency of forming functional chimeric kidneys using transplanted metanephroi is a key improvement.

The following theory, by which the present inventors are not bound, explains why certain sites may be superior in terms of kidney development, either in growth of transplanted kidney metanephroi and/or in formation of a functional chimeric kidney capable of collecting urine.

Vascular supply through the hilum is well known in kidney anatomy, but is not mentioned as being important or relevant in prior art relating to chimeric kidney formation. It is likely that the blood supply for a transplanted metanephros will tap from the arterial system and feed into the venous system. It is therefore likely that metanephroi transplanted close to an artery (e.g. renal artery) will have a shorter supply and a longer return (to use a plumbing analogy) while metanephroi transplanted close to a large vein will have a longer supply and a shorter return. If a supply taps from a major (or large) artery, this will resemble the physiologic development of normal kidneys. See Fig. 14 and Fig. 15.

In the present invention, microvasculature for transplanted metanephros development may be directed through the hilum for efficient filtration. The normal adult metanephros (kidney) is kidney-bean shaped, with the indent being called the hilum. The hilum is the region of the kidney where the renal artery and nerves enter and the ureter and renal vein exit. The renal pelvis is the region immediately inside the hilum. A connective tissue capsule surrounds the periphery of the kidney. Beneath the capsule is a region called the cortex. The cortex is where the renal corpuscles (glomeruli and Bowman's capsules) and most of the nephric tubules are located. A portion of the nephric tubule dips down below the

cortex into a region known as the medulla. The medulla does not have any renal corpuscles, but is composed of nephric tubules, collecting ducts and numerous capillaries. The medulla narrows into a pyramid-shaped structure, known as the renal papilla, near the renal pelvis. Urine is discharged from the collecting ducts, which open into the renal papilla, and moves through the renal papilla to the renal pelvis and out of the kidney through the ureter.

In a metanephroi transplanted to the omentum (Hammerman's preferred site), the blood supply is from various diffuse sources within the omentum. Although all these vessels do enter the metanephroi through the hilum, this is supplied via a number of disparate smaller vessels (all supplied from the gastro-epiploic artery, itself a branch of the caeliac artery which is itself a branch of the aorta) rather than tapped from a large blood vessel.

In a metanephroi transplanted to the kidney capsule (Hammerman's other preferred site), the blood supply taps from within the adult kidney. This correlates with poorer kidney development.

The present inventors have observed that when transplanted metanephroi are allowed to grow in the recipient, a small area of graft-derived fat is deposited around the hilum, i.e. where the ureter enters the metanephroi (see for example in Fig. 11A). This area of fat could be useful for orienting the ureter during connection of the ureter to the recipient's ureter or bladder.

The inventors noted that when dissecting metanephroi from the embryos, most of the capsule comes away cleanly from surrounding tissue. We believe that it is likely that some mesenchymal stem cells or other precursors from the area around the hilum grow into graft-derived fat described above.

Claims

1. A chimeric kidney located on or near a large blood vessel in the abdomen of a mammalian host, in which the chimeric kidney is capable of producing urine.
2. The chimeric kidney according to claim 1, in which the chimeric kidney is located in the peritoneal cavity of the host.
3. The chimeric kidney according to either of claim 1 or claim 2, in which the chimeric kidney is in fluid communication with the host's large blood vessel via the hilum of the chimeric kidney.
4. The chimeric kidney according to any preceding claim, in which the chimeric kidney is in fluid communication with the host's large blood vessel via vasculature which originates at the large blood vessel and which enters the chimeric kidney within an area defined by a frustoconical shape having a cone angle of less than 90 degrees and an apex at the chimeric kidney.
5. The chimeric kidney according to any preceding claim, in which the chimeric kidney has developed a ureter to facilitate externalisation of urine.
6. The chimeric kidney according to any preceding claim, in which urine produced by the chimeric kidney is excreted via the host's ureter.
7. The chimeric kidney according to any preceding claim, in which the chimeric kidney is formed from an embryonic metanephros.
8. The chimeric kidney according to claim 7, in which the embryonic metanephros is porcine.

9. The chimeric kidney according to any preceding claim, in which the host is human.
- 5 10. The chimeric kidney according to any preceding claim, in which the large blood vessel is the renal vein.
11. The chimeric kidney according to any of claims 1 to 9, in which the large blood vessel is the renal artery.
- 10 12. A chimeric kidney multiplex comprising two or more chimeric kidneys as defined in any of claims 1 to 11.
13. The chimeric kidney multiplex according to claim 12, in
15 which the chimeric kidney comprises an interconnecting manifold linking the two or more chimeric kidneys.
14. A method of increasing the nephron mass of a mammalian recipient comprising implanting a metanephros of an
20 embryonic mammalian donor on or near a large blood vessel of the recipient under conditions that allow the metanephros to become vascularised.
15. The method according to claim 14, in which the large
25 blood vessel is located in the peritoneal cavity.
16. The method according to either of claim 14 or claim 15, in which the vascularised metanephros forms a chimeric
kidney that produces urine and develops a ureter that
30 facilitates externalisation of the urine.
17. The method according to any of claim 14 to claim 16, in which the large blood vessel is the aorta or a branch of the
aorta.

18. The method according to claim 17, in which the large blood vessel is a renal artery, an iliac artery, a gonadal artery or an hepatic artery.

5 19. The method according to either of claim 15 or claim 16, in which the large blood vessel is the vena cava or a branch of the vena cava.

10 20. The method according to claim 19, in which the large blood vessel is a renal vein, an iliac vein, a gonadal vein or an hepatic vein.

15 21. The method according to either of claim 15 or claim 16, comprising the steps of making a surface abrasion on or near a superior lobe of a liver of the recipient and implanting the metanephros on or near the abrasion to allow the metanephros to connect to an hepatic blood supply.

20 22. The method according to any of claims 14 to 21, in which the metanephros has an intact renal capsule.

25 23. The method according to any of claims 14 to 22, in which at least two whole metanephroi, each with renal capsules intact, are implanted into the recipient.

24. The method according to any of claims 14 to 23, in which the metanephros is allogeneic to the recipient.

30 25. The method according to any of claim 14 to 23, in which the metanephros is xenogeneic to the recipient.

35 26. The method according to claim 25, in which the metanephros is derived from a non-human (for example porcine) embryonic mammalian donor.

27. The method according to claim 26, in which the porcine donor is free of zoonoses.

28. The method according to any of claims 14 to 27, further comprising immunosuppressing the recipient.

5 29. The method according to any of claims 14 to 28, in which the metanephros is obtained from the donor within 2 to 4 days after embryonic development of the metanephros begins.

10 30. The method according to any of claims 14 to 29, in which the metanephros is obtained from the donor prior to the presence of significant blood vessels within the metanephros.

15 31. The method according to any of claims 14 to 30, in which the recipient has reduced functional renal mass prior to implantation of the metanephros.

20 32. The method according to any of claims 15 to 31, in which after the ureter of the chimeric kidney develops, a ureter to ureter anastomosis is performed to provide fluid communication between the ureter of the chimeric kidney and a ureter of the recipient.

25 33. The method according to any of claims 15 to 31, in which after the ureter of the chimeric kidney develops, a ureter to bladder anastomosis is performed to provide fluid communication between the ureter of the chimeric kidney and the bladder of the recipient.

30 34. The method according to claim 33, wherein the metanephros is implanted on or near the iliac vein or iliac artery.

35 35. The method according to any of claims 15 to 31, the recipient's ureter is connected (for example, anastomosed)

to a cyst (for example, containing urine) located around the ureter developed by the chimeric kidney.

5 36. The method according to any of claims 14 to 35, in which two or more metanephroi are implanted into the recipient.

10 37. The method according to claim 36, in which the two or more embryonic metanephroi are linked using an interconnecting manifold.

38. The method according to any of claims 14 to 37, in which the mammalian recipient is a juvenile or adult.

15 39. The method according to any of claims 14 to 38, in which the metanephros is implanted within five hours, preferably 2 to 4 hours, after removal from the embryonic donor.

20 40. The method according to any of claims 14 to 39, in which prior to implantation of the metanephros, renal tissue is removed from the mammalian recipient.

25 41. The method according to any of claims 14 to 40, in which the metanephros is transplanted to a site within a recipient and connected to the large blood vessel via a tube or canula.

30 42. A chimeric kidney obtainable using the method of any of claims 14 to 41.

35 43. A method of growing a donor embryonic mammalian metanephros in a mammalian recipient, comprising the step of implanting the metanephros on or near a large blood vessel of the recipient under conditions that allow the metanephros to become vascularised.

44. A method of reducing the inflammatory response following implantation of a donor embryonic mammalian metanephros in a mammalian recipient, comprising the step of implanting the metanephros on or near a large blood vessel of the recipient under conditions that allow the metanephros to become vascularised.
- 5

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Fig. 1

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Fig. 2A

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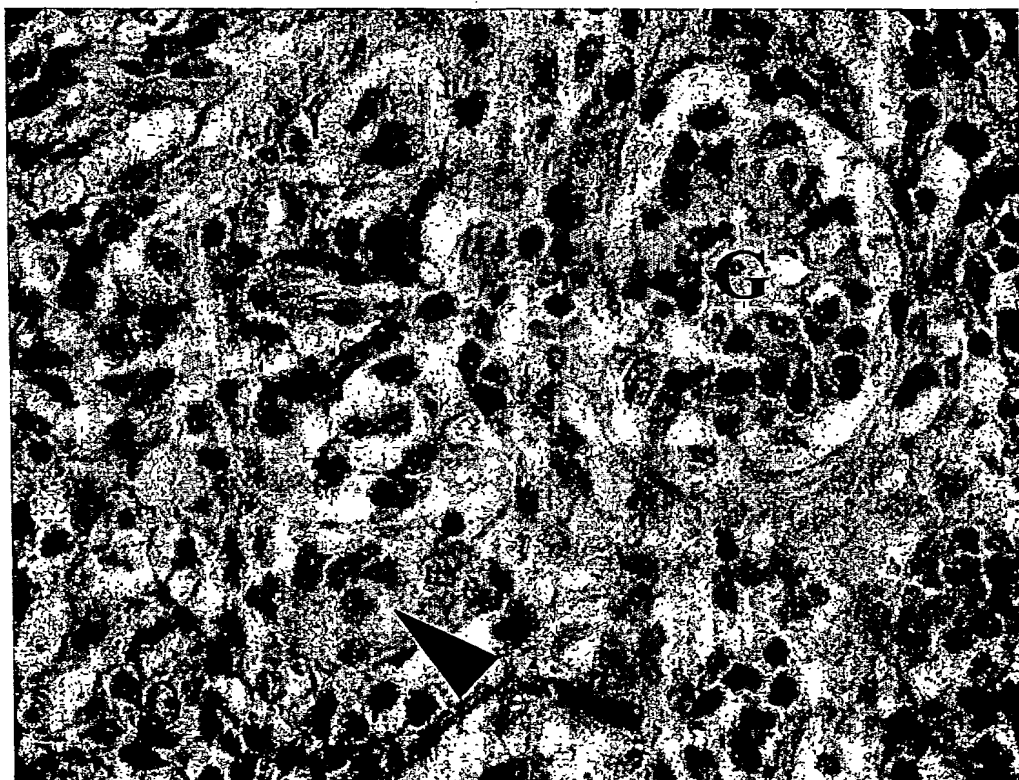


Fig. 2B

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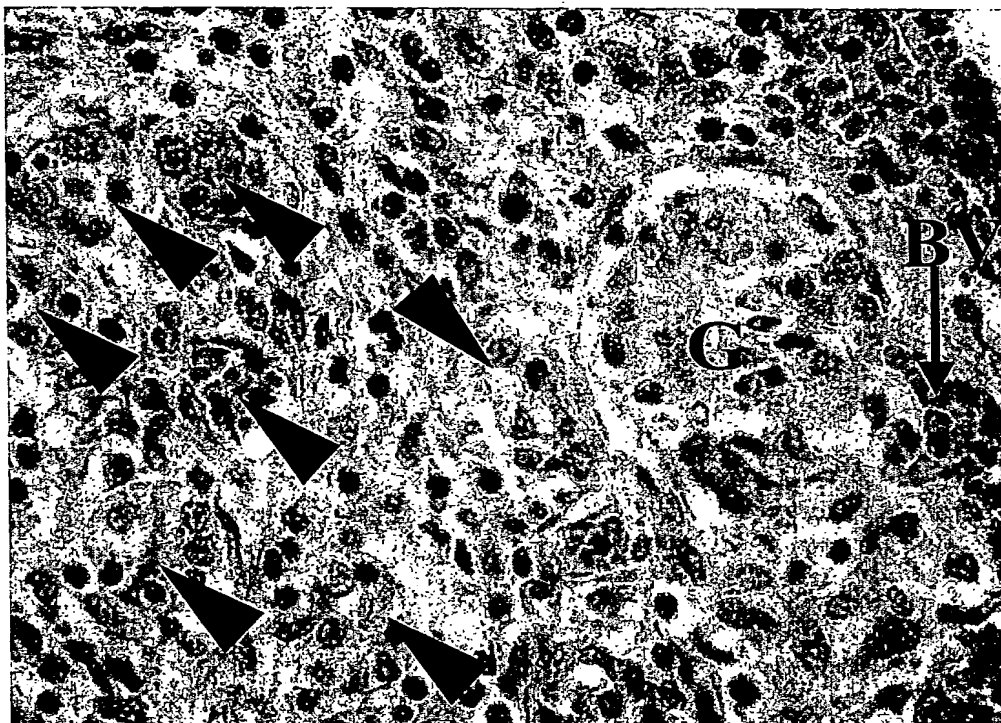


Fig. 2C

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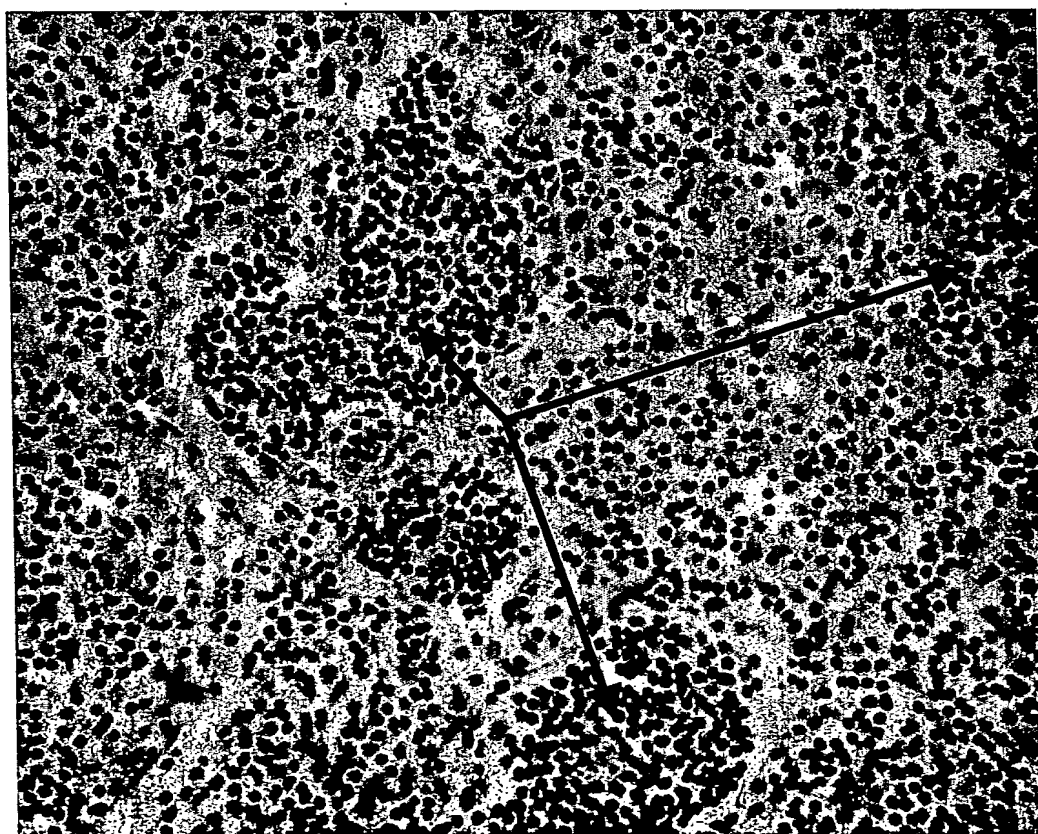


Fig. 3A

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Fig. 3B

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Fig. 4A

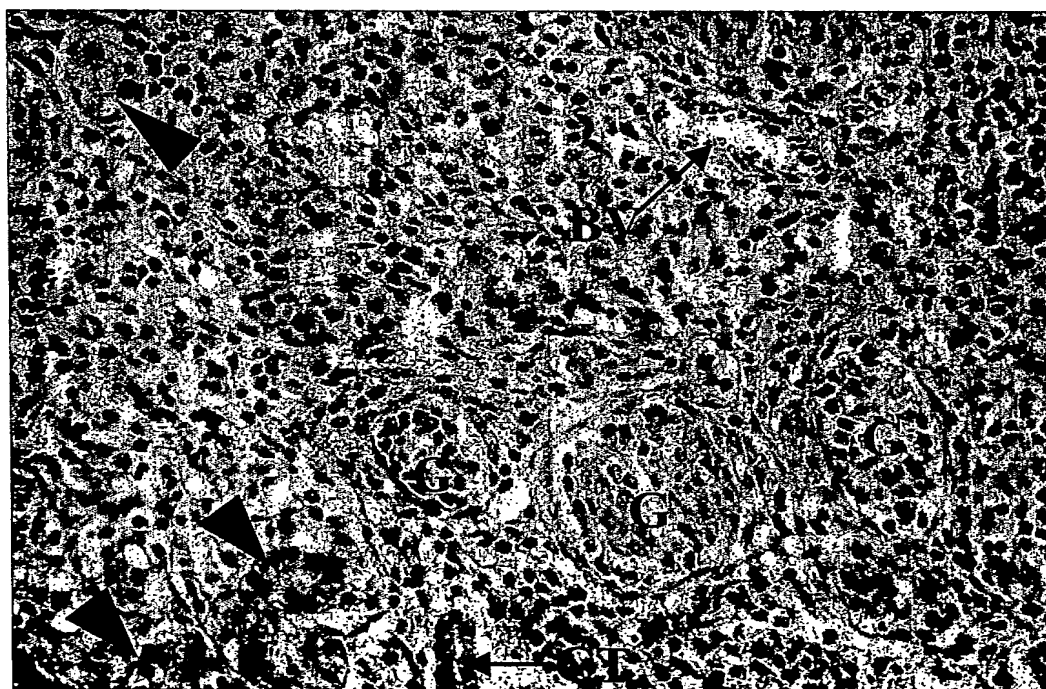


Fig. 4B

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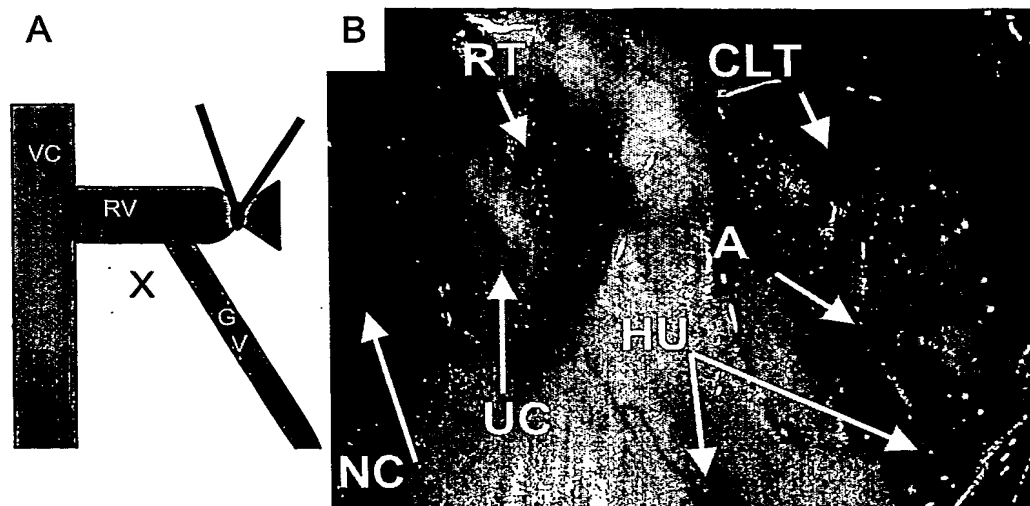


Fig. 5

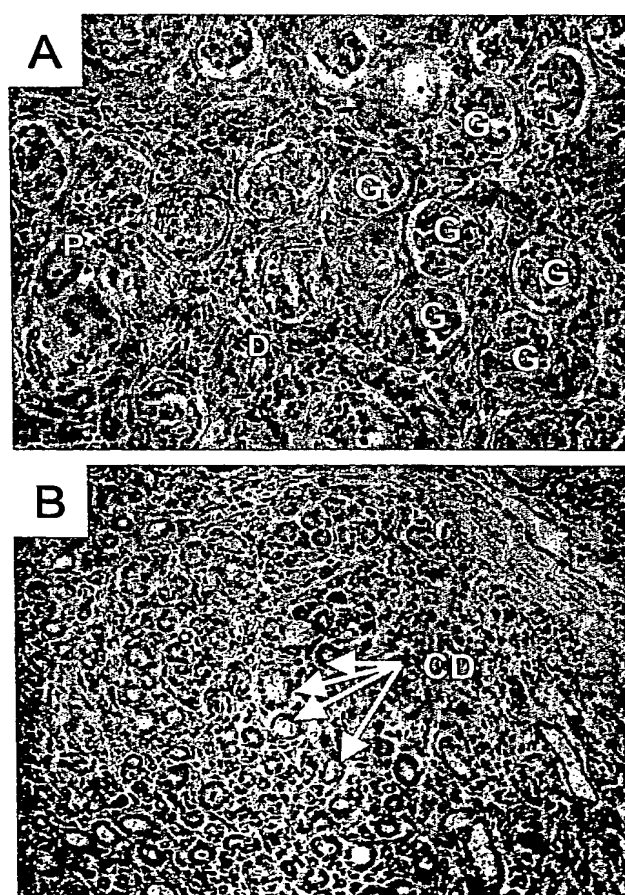


Fig. 6

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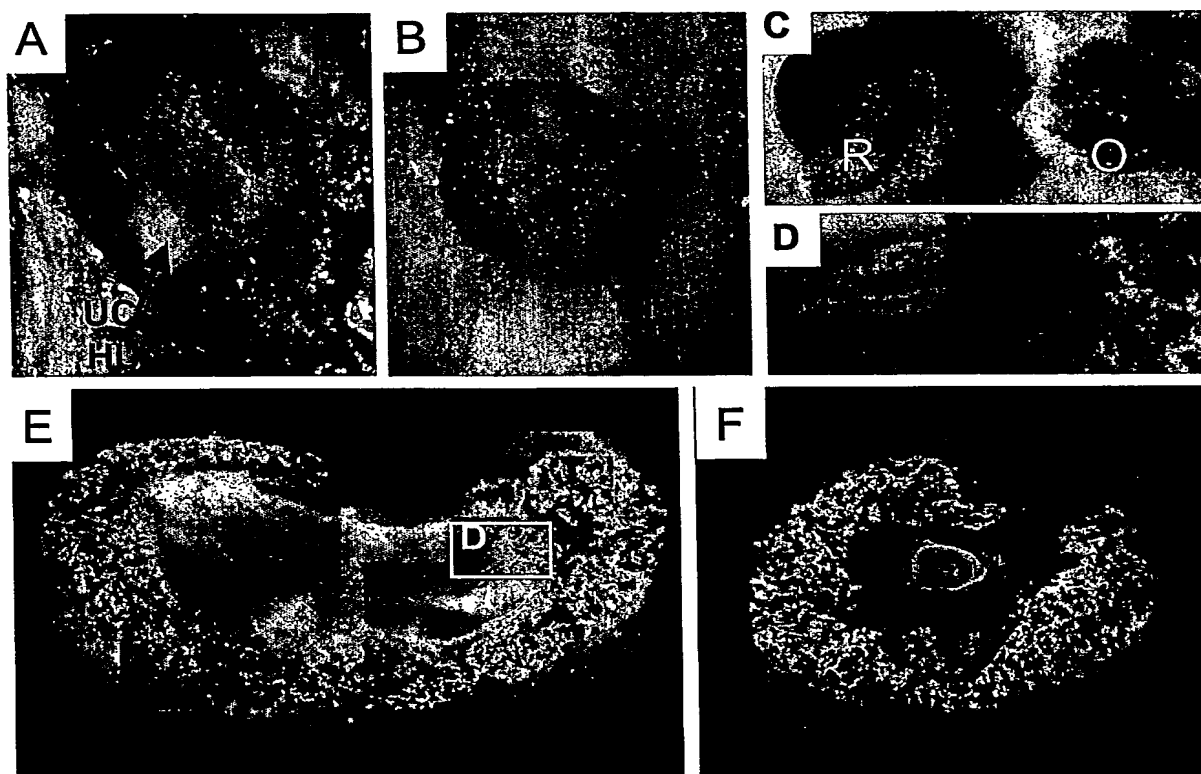


Fig. 7

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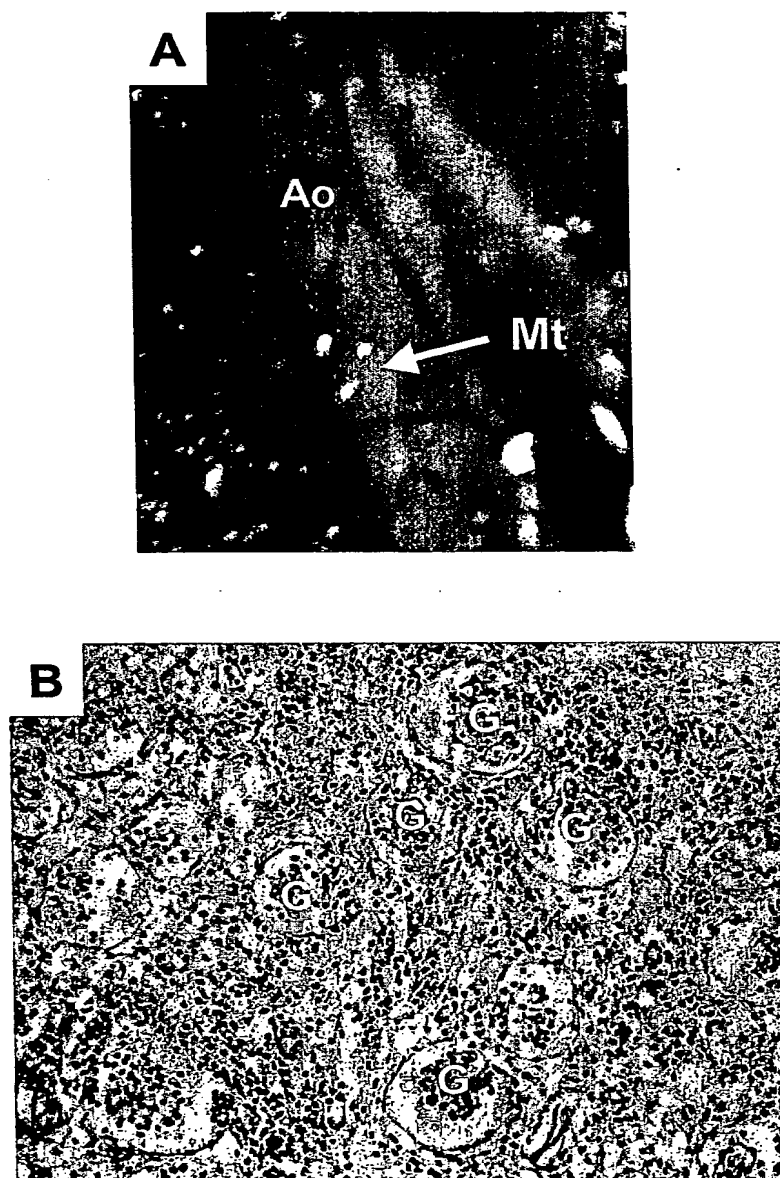


Fig. 8

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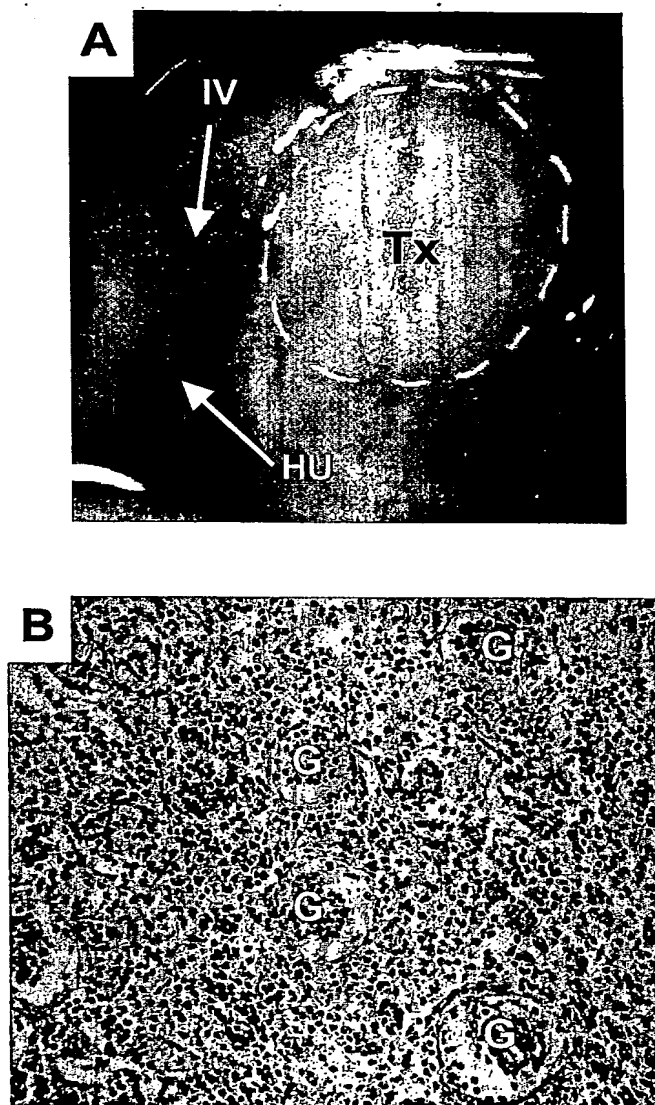


Fig. 9

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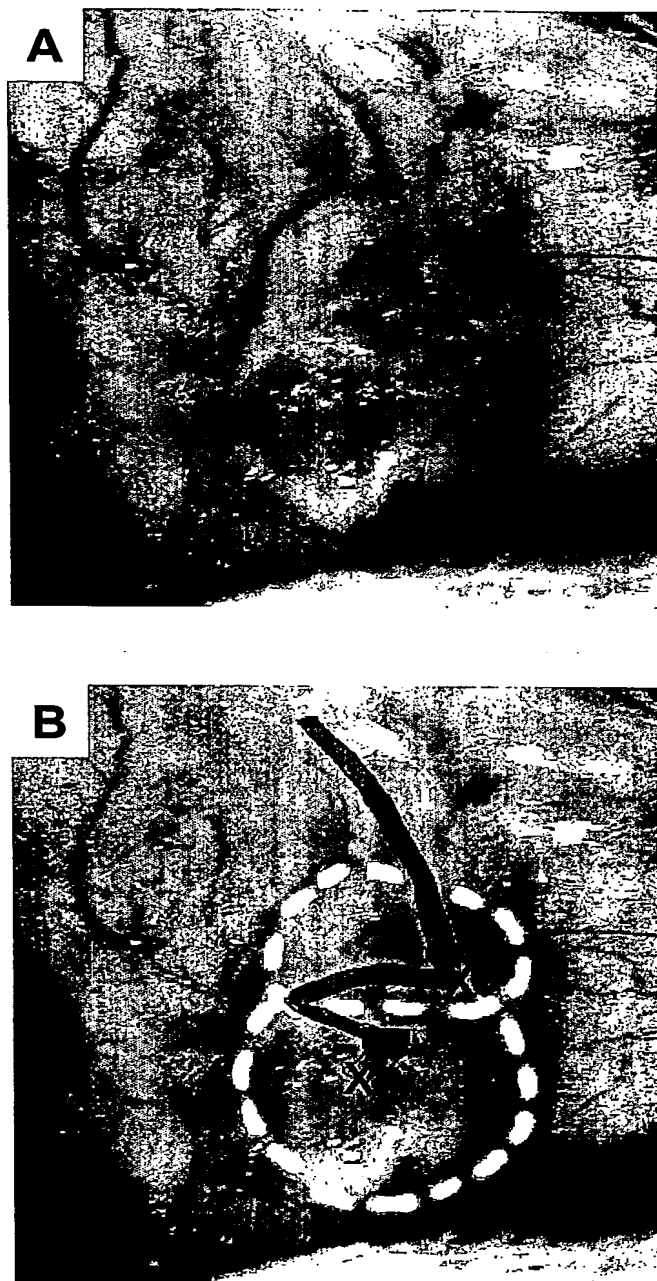


Fig. 10

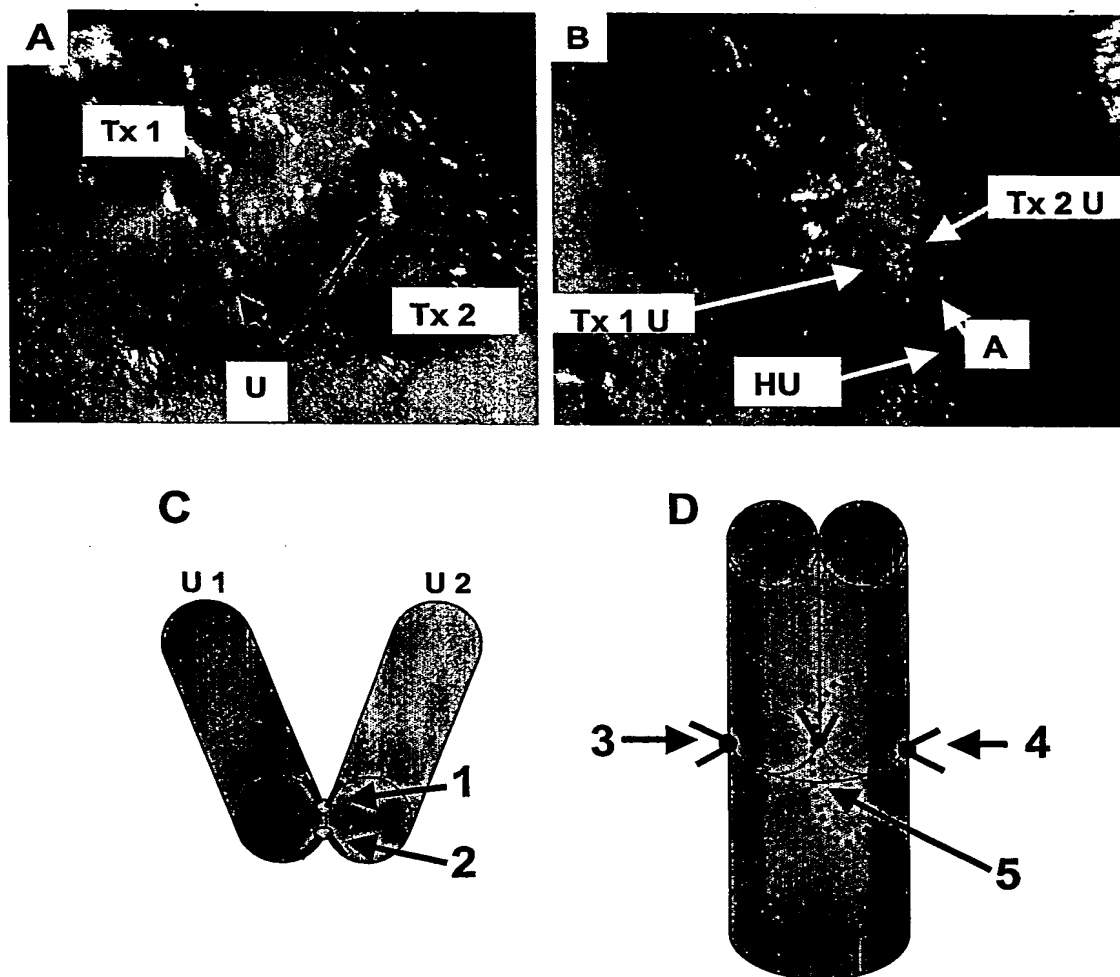


Fig. 11



Fig. 12

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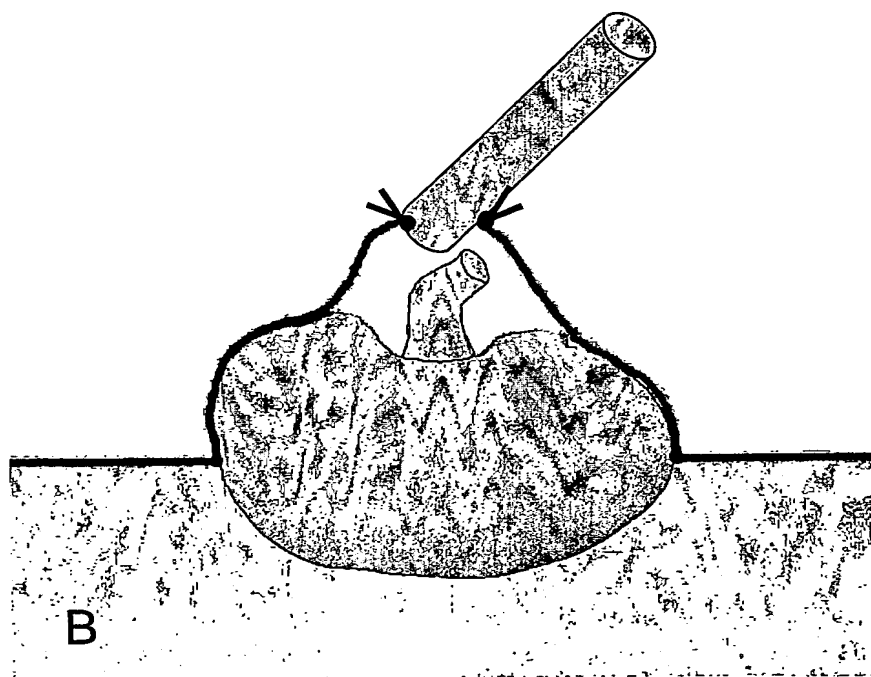
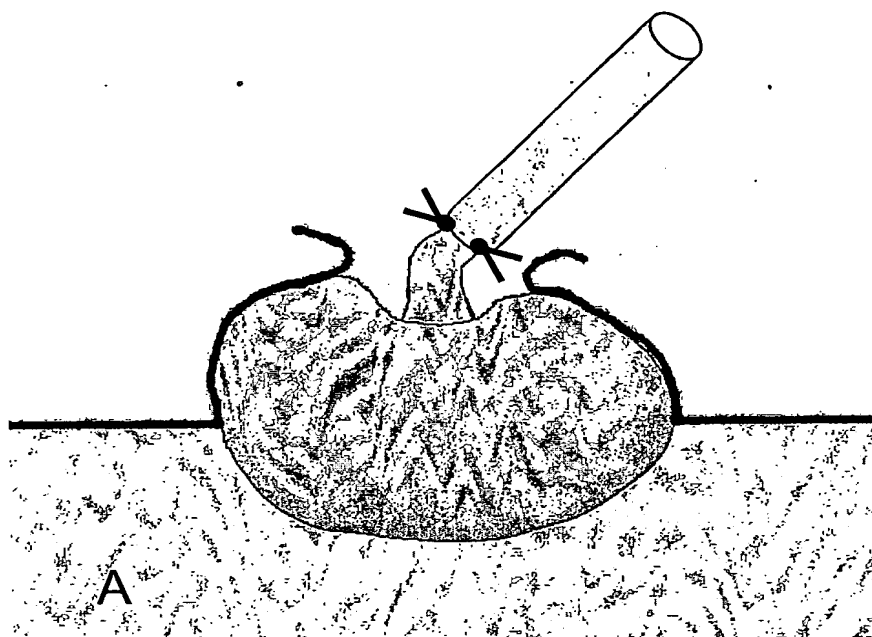


Fig. 13

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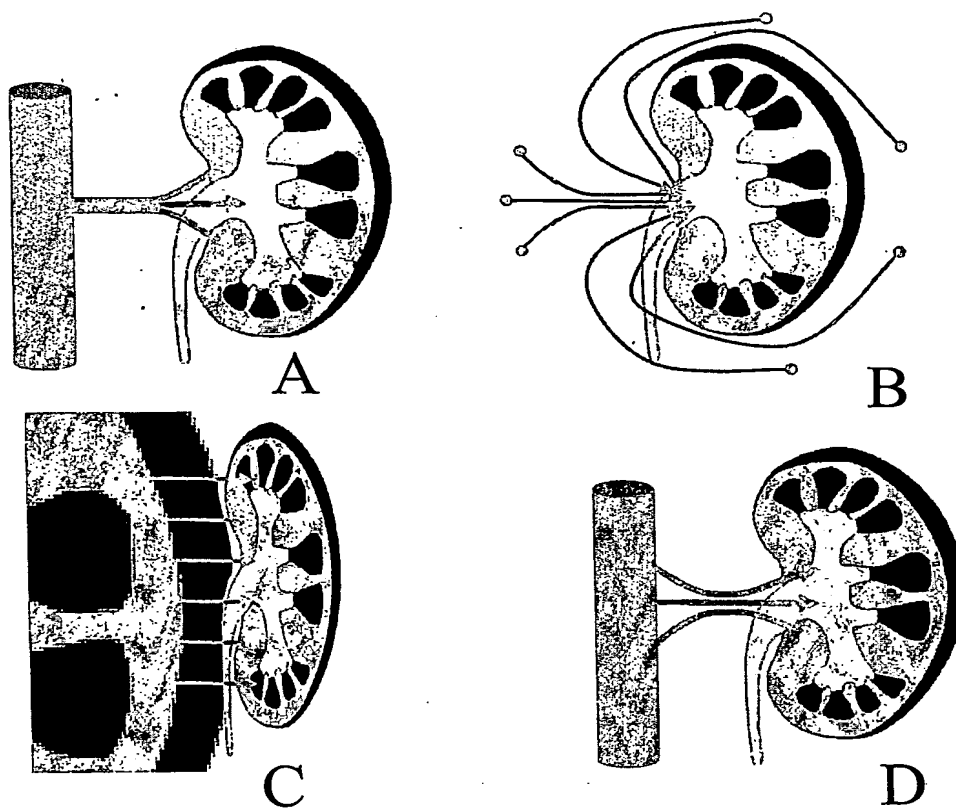


Fig. 14

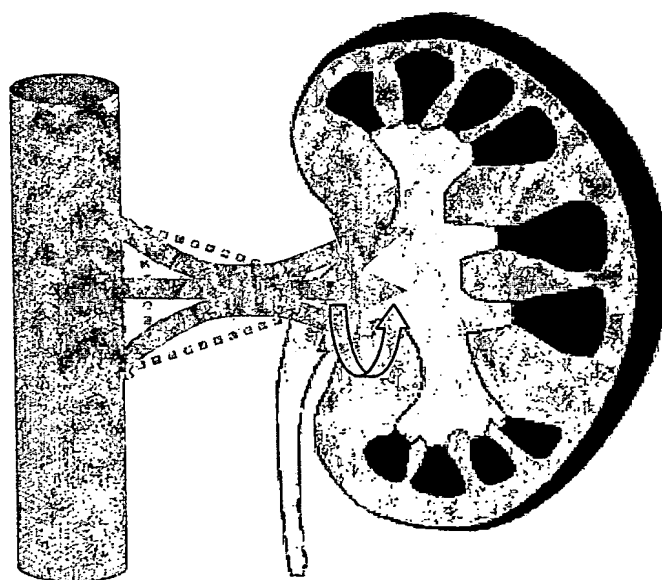


Fig. 15

INTERNATIONAL SEARCH REPORT

International Application No
PC 03/03575

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/23 A61P13/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, PASCAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 976 524 A (HAMMERMAN MARC) 2 November 1999 (1999-11-02) cited in the application cited in the application column 3, line 49 - line 59 column 4, line 18 - line 57 column 5, line 15 - column 6, line 65 claims 1-16	1-44
X	EP 0 853 942 A (UNIV WASHINGTON) 22 July 1998 (1998-07-22) claims 1-27 the whole document	1-44

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 October 2003

Date of mailing of the international search report

17/11/2003

Name and mailing address of the ISA

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Authorized officer

Fayos, C

INTERNATIONAL SEARCH REPORT

International Application No
PC 03/03575

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 41713 A (ROGERS SHARON A.; UNIV WASHINGTON (US); HAMMERMAN MARC R (US)) 20 July 2000 (2000-07-20) page 6, line 5-29 page 11, line 21 -page 17, line 12 claims 1-14	144
X	ROGERS S A ET AL: "Transplantation of rat metanephroi into mice." AMERICAN JOURNAL OF PHYSIOLOGY. REGULATORY, INTEGRATIVE AND COMPARATIVE PHYSIOLOGY. UNITED STATES JUN 2001, vol. 280, no. 6, June 2001 (2001-06), pages R1865-R1869, XP002259735 ISSN: 0363-6119 Abstract; Discussion	1-44
X	HAMMERMAN M R: "TRANSPLANTATION OF RENAL PRECURSOR CELLS: A NEW THERAPEUTIC APPROACH" PEDIATRIC NEPHROLOGY, SPRINGER VERLAG, BERLIN, DE, vol. 14, no. 6, June 2000 (2000-06), pages 513-517, XP000929752 ISSN: 0931-041X abstract page 513, column 2, paragraph 3 -page 514, column 1, paragraph 5 page 515, column 1, paragraph 4 -column 2, paragraph 2	1-44
X	ROGERS S A ET AL: "TRANSPLANTATION OF DEVELOPING METANEPHROI INTO ADULT RATS" KIDNEY INTERNATIONAL, NEW YORK, NY, US, vol. 54, no. 1, July 1998 (1998-07), pages 27-37, XP000929746 ISSN: 0085-2538 Abstract, Discussion	1-44
X	HAMMERMAN M R: "NEW DEVELOPMENTS IN KIDNEY DEVELOPMENT" NEPHRON, XX, XX, vol. 81, no. 2, 1999, pages 131-135, XP000929750 ISSN: 0028-2766 abstract page 132, column 1, paragraph 2 page 135, column 1, paragraph 3 -column 2, paragraph 2	1-44

INTERNATIONAL SEARCH REPORT

In. 1st Application No

PCT 03/03575

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROGERS S A ET AL: "Transplantation of metanephroi after preservation in vitro." AMERICAN JOURNAL OF PHYSIOLOGY. REGULATORY, INTEGRATIVE AND COMPARATIVE PHYSIOLOGY. UNITED STATES AUG 2001, vol. 281, no. 2, August 2001 (2001-08), pages R661-R665, XP002259736 ISSN: 0363-6119 Abstract, Discussion	1-44
X	HAMMERMAN M R: "Growing kidneys." CURRENT OPINION IN NEPHROLOGY AND HYPERTENSION. ENGLAND JAN 2001, vol. 10, no. 1, January 2001 (2001-01), pages 13-17, XP009020203 ISSN: 1062-4821 the whole document	1-44
X	HAMMERMAN MARC R: "Xenotransplantation of renal primordia." CURRENT OPINION IN NEPHROLOGY AND HYPERTENSION. ENGLAND JAN 2002, vol. 11, no. 1, January 2002 (2002-01), pages 11-16, XP009020207 ISSN: 1062-4821 the whole document	1-44
P,X	HAMMERMAN MARC R: "Tissue engineering the kidney." KIDNEY INTERNATIONAL. UNITED STATES APR 2003, vol. 63, no. 4, April 2003 (2003-04), pages 1195-1204, XP001155987 ISSN: 0085-2538 page 1198, column 2, paragraph 1 -page 1202, column 1, paragraph 1 page 1203, column 1, paragraph 7 -page 1203, column 2, paragraph 2	1-44
P,X	HAMMERMAN MARC R: "Xenotransplantation of developing kidneys." AMERICAN JOURNAL OF PHYSIOLOGY. RENAL PHYSIOLOGY. UNITED STATES OCT 2002, vol. 283, no. 4, October 2002 (2002-10), pages F601-F606, XP002259738 ISSN: 0363-6127 abstract page F602, column 2, paragraph 2 -page F605, column 2, paragraph 5 --- -/--	1-44

INTERNATIONAL SEARCH REPORT

International Application No

PO 03/03575

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT.

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	HAMMERMAN MARC R: "Therapeutic promise of embryonic kidney transplantation." NEPHRON, vol. 93, no. 2, February 2003 (2003-02), pages e58-e62, XP000902020 ISSN: 0028-2766 (ISSN print) page 4	1-44
P,X	ROGERS SHARON A ET AL: "Transplantation of pig metanephroi." ASAIO JOURNAL (AMERICAN SOCIETY FOR ARTIFICIAL INTERNAL ORGANS: 1992) UNITED STATES 2003 JAN-FEB, vol. 49, no. 1, January 2003 (2003-01), pages 48-52, XP009020205 ISSN: 1058-2916 the whole document	1-44
A	LOUGHNA S ET AL: "A molecular and genetic analysis of renalglomerular capillary development." ANGIOGENESIS. NETHERLANDS 1997, vol. 1, no. 1, 1997, pages 84-101, XP009020201 ISSN: 0969-6970 the whole document	1-44
A	CLARK A T ET AL: "Molecular regulation of nephron endowment." THE AMERICAN JOURNAL OF PHYSIOLOGY. UNITED STATES APR 1999, vol. 276, no. 4 Pt 2, April 1999 (1999-04), pages F485-F497, XP002259739 ISSN: 0002-9513 the whole document	1-44

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/03575

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-44
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-44 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 1-44.

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

INTERNATIONAL SEARCH REPORT

In **national Application No**
PCT **03/03575**

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